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Research Article

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Evaluation of Culture and Real Time PCR Methods for the Diagnosis of Brucellosis

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ABSTRACT

This study aimed to determine the presence of *Brucella* spp. in 73 abortion materials collected from various provinces of Turkey. Culture isolation and Real-time PCR methods were used for this purpose, and the laboratory diagnosis was compared. The clinical samples were taken from aborted cattle. A total of 79 samples were used for this study, consisting of 28 fetal stomach contents and 51 vaginal swabs. The presence of *Brucella* spp. was investigated using both culture isolation and real-time PCR methods. Of the collected samples, eight (10.12%) were found to be positive for *Brucella* spp. through culture isolation. These same samples were then subjected to Real Time PCR testing for comparison. Thirteen point nine two percent (11/79) of the samples tested positive for *Brucella* spp. using real-time PCR. This suggests that inhibitors, bacterial load in clinical samples, and possible contamination may reduce the chance of isolating the bacteria in culture or lead to false negative results. Therefore, it can be concluded that real-time PCR is a fast and reliable alternative to culture for diagnosing brucellosis.

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INTRODUCTION

Brucellosis is an important and widespread zoonotic disease in terms of public health, causing economic losses in Turkey and in the world. This zoonosis, which affects our country, continues to be updated within the scope of one health. In the eradication of brucellosis, it is very important to immediately intervene in the diseased area and diagnose it. In this way can be protected human and animal health and risks can be minimized. One of the diagnostic methods used in diagnosing the disease of brucellosis, isolation from culture is a time-consuming, difficult method that can give false negative results. Therefore, molecular methods have become increasingly important nowadays (Probert, 2004).

Brucellosis is caused by gram-negative aerobic coccobacilli, also known as facultative intracellular bacteria. Identification of *Brucella* spp. involves culture isolation, biochemical tests, serological tests, and molecular methods (Baysal, 1989; Güzelant, 2009; Jimenez, 2004; Qasem, 2015). This process is time-consuming and tedious, and the culture isolation stage poses a high risk of infection for laboratory workers (Qasem, 2015). In humans, brucellosis can present with a range of clinical symptoms. Acute cases may exhibit fever, myalgia, myocarditis, and pancytopenia (Özenci, 1984; Qasem, 2015; Ulaş, 2012). The disease can progress to a chronic state, affecting various organs and tissues, particularly the joints (Probert, 2004).

To overcome these problems, nucleic acid amplification remains up-to-date for rapid detection of *Brucella* and validation of tests. Alternative methods such as real time PCR, which provides rapid and reliable diagnosis in brucellosis eradication, have always started a desired process (Probert, 2004).

Brucellosis diagnosis involves culture isolation, serological tests, and molecular studies. Although serological tests are commonly used, they may not be sufficient for early-stage diagnosis and may not differentiate between active and inactive infections due to cross-reactions (Diaz, 2020; Ko, 2012). Culture isolation is considered the gold standard for brucellosis diagnosis. The most commonly used methods are those described by Elfaki (2005). However, when attempting to isolate brucellosis from culture, contamination with flora is a frequent occurrence, particularly in environmental samples. Given the laboratory conditions, high-risk factors require increased attention, as noted by Yagupsky (1999).

For this reason, different diagnostic methods have been developed. For the diagnosis of brucellosis, DNA-based methods such as polymerase chain reaction (PCR) are used especially for confirmation. There are few reports on the comparison of these tests. The aim of the current study is to compare the gold standard culture method, which is frequently used in different brucellosis tests, with the real time PCR method.

The aim of this study is to determine the presence of Brucellosis disease, which has a high zoonotic importance, by culture and real-time PCR. At the same time, these methods will be evaluated comparatively.

MATERIAL AND METHOD

The ethical permission was ensured from The Local Ethics Committee code of 2021/1 and 17.02.2021.

Study Area and Sample Collection

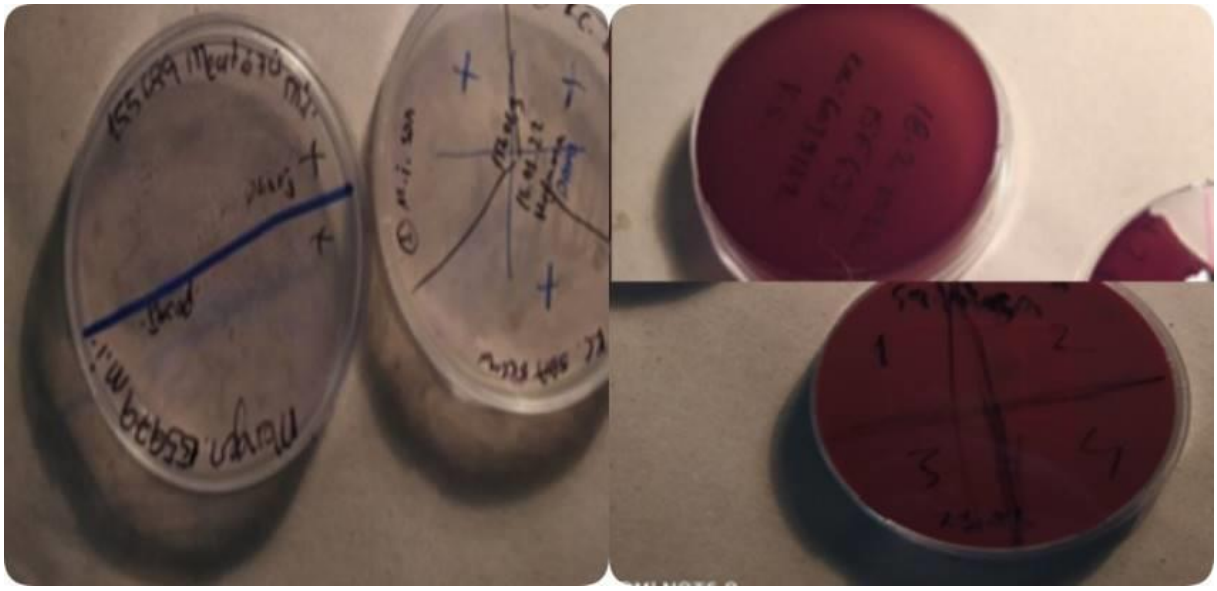
The study was conducted in Central Anatolia and the Western Black Sea province of Turkey. A total of 79 clinical materials were collected from different settlements in Turkey, including 28 fetal stomach contents and 51 vaginal swabs from aborted cattle patients. The clinical samples were stored at -20°C for culture analysis and RT-PCR.

Culture Analysis

Skirrow agar and SDA were used for culture isolation of *Brucella* spp. After inoculation, the medium was incubated at 37°C with a microaerophilic environment for 1-3 weeks. After the culture isolation, the species started to breed from the 3rd day and daily controls were provided. Colony morphologies in the passages of the samples were observed (Kurtoğlu, 2014; Marouf;2021). Confirmed by Gram stain, biochemical tests oxidase tests. catalase and urease activity (Mena-Bueno, 2021; Yagupsky, 2022). After isolation, macroscopic morphology, incubation time and biochemical properties of colonies were tested to confirm *Brucella* spp (Mena-Bueno, 2021). Successful colonies obtained were stored in stock solution at -80°C (Yagupsky, 2022).

Figure 1.

Image of Culture Isolation Media for Brusella spp. (SDA and Skirrow Medium)



Real Time PCR

When performing DNA extraction from our materials, it was prepared in accordance with the manufacturer's instructions. Nucleic acid purification kit (QIAampDNA Mini Kit, Qiagen, Germany) was used for this procedure.

The primer and probe sequence used for the TaqMan real-time PCR assay for identification of *Brucella* spp. It targets the bcsp31 gene (Sabour et al, 2020).

The conditions of the real-time PCR test were performed as follows: 40 cycles of 95 °C for 10 minutes, 95 °C for 20 seconds, and 56 °C for 45 seconds. Oligonucleotide primers and probes used in real-time PCR method to detect *Brucella* spp agent in our samples; Forward primer:

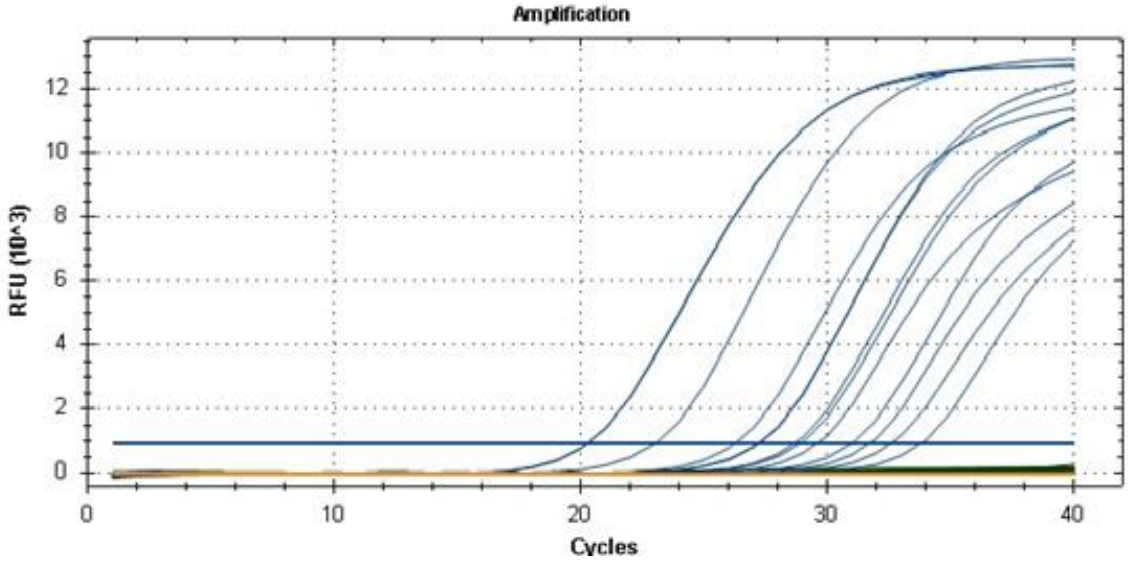
GTCCGGTTGCCAATATCAATGC, Reverse primer: GGGTAAAGCGTCGCCAGAAG Reverse: AAATCTTCCACCTTTGCCCTTGCCATCA (Sabour et al, 2020).

RESULTS

After isolating clinical materials from culture, it was found that eight (10.12%) samples contained *Brucella* spp. In order to eliminate Salmonella spp and Campylobacter spp, inoculations were made on MacConkey agar and Skirrow agar, but the results were negative. Real Time PCR test method identified 11 (13.92%) samples as *Brucella* spp (Figure 2).

Figure 2.

Brusella spp. Amplification Curves of Real Time PCR Reactions of Clinical Materials



DISCUSSION

Control and eradication methods are crucial for both animals and humans. Vaccination and eradication of farm animals are particularly important. Vaccines are highly effective in immunizing farm animals against diseases. The control method used for brucellosis in our country and around the world is vaccination of animals. However, animal movements and uncontrolled contact of breeders with farm animals can lead to the spread of diseases. Due to its zoonotic nature, effective antibiotic treatment for humans is often challenging when dealing with brucellosis (Hoffman, 2016). Therefore, controlling animal brucellosis and, quick and accurate diagnosis of the disease is crucial in preventing human infection.

Traditional bacteriology methods can be very demanding and long-lasting (Probert, 2004). Various media such as Columbia blood agar, Serum dextrose agar, skirrow agar, tryptone soy agar, serum dextrose agar and *Brucella* medium base are used to isolate *Brucella* species (De Miguel, 2011). In our routine laboratory, *Brucella* spp. we get effective results from especially two media that we use in the diagnosis of the disease. Therefore, *Brucella* spp. serum dextrose agar (SDA) (Oxoid) and Skirrow agar (Oxoid) were used to detect the causative agent. All samples were processed under Biosafety Level Two (BSL-2). Particularly high personal security procedures have been implemented. In addition to these measures, staff training, decontamination, record keeping, emergency procedures and risk assessments have been completed (Schuring, 1991). Successful isolation of *Brucella* spp.

from clinical samples may vary depending on the level of environmental contamination and bacterial load. Therefore, it is important to pay special attention to potential contaminants when working in laboratory conditions to increase the chances of successful isolation. In our study, *Brucella* was successfully isolated from eight clinical samples. The real-time PCR results for the same samples were positive. Furthermore, the samples were evaluated for *Salmonella* and *Campylobacter* species using the culture method, but all samples tested negative. In addition to the eight samples that tested positive in culture, three more samples (two vaginal swabs and one fetal stomach content) were found to be positive for *Brucella* spp by real-time PCR. Based on the anamnesis and positive real-time PCR results, these three animals were classified as 'probable patients' due to abortion.

Today, molecular methods are frequently used. Among these methods, Real-Time PCR method, sensitivity, amplification efficiency, results in a short time, it is known to be more advantageous than other methods (Bounaadja, 2009; Probert, 2004). Culture analysis and real-time methods were compared in research on humans and animals at different studies (Dal, 2019). In these comparisons, sensitivity and specificity studies were carried out. In most studies by researchers PCR was observed to be more effective than isolation from culture (Abedi, 2020; El-Diasty, 2016). On the other hand, real time PCR played a more effective role in detecting the presence of DNA.

Our research compared the results of the real-time PCR method with the culture method, confirming that real-time PCR is a viable alternative for diagnosis, as previously shown in other studies (Awwad, 2016; Mukherjee, 2015; Navarro, 2002). Given the high cost and difficulty of vaccination/culling studies against this infection, which is currently prevalent in our country, accurate diagnosis is critical for effective control. Fast and sensitive diagnostic tools are crucial for developing an emergency protection and control strategy against brucellosis, particularly during disease outbreaks when animals are experiencing abortions (Mukherjee, 2015). Additionally, this is a preventive measure for public health within the framework of One Health.

As a result, as emphasized in the research articles, it was observed in my study that the real time PCR test was very fast and reliable as a method in the diagnosis of the agent. In the light of all this information, real-time PCR can be used as an alternative method to isolation from culture, which has disadvantages in terms of time control and workload in the diagnosis of brucellosis disease.

Ethical Statement

A section of this study was presented as Oral Presentation and Abstract Paper at the Second International Congress on Biological and Health Science on 24.02.2022.

Ethics Committee Approval

17/01/2021 dated 17/02/2021 and 2021/01 numbered was given by Veterınary Control Central Research Instıtute Local Ethics Committee

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Conflict of Interest

Authors declare that there is no conflict of interest.

Sustainable Development Goals (SDG): 3 Good Health and Well-Being

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Comparison of Serum and Plasma Aluminum Concentrations in Sheep

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ABSTRACT

Although Aluminum (Al) is a non-essential toxic (neurotoxic, immunotoxic, cardiotoxic, etc.) element, it is widely used in several industries (pharmaceuticals, cosmetics, food, etc.). Al-containing vaccines, medicines, contaminated food, and polluted air are the main routes of exposure (inhalation, dermal or oral). Determination and monitoring of blood element concentrations is an important tool for the clinical diagnosis of toxicity in animal health and sheep is one of the indicator species for assessing environmental health status. Aluminum is highly (>80) bound to protein in blood. Inductively coupled plasma mass spectrometry (ICP-MS) is a current analytical method with high potential to contribute to clinical diagnosis in the field of animal health, where elemental concentrations in biological samples can be measured at multiple and parts per trillion (ppt) concentrations. The aim of this study was to determine, statistically analyze, and compare Al concentrations in sheep (n=12) serum and plasma by ICP-MS. The concentrations (mean±SE) were 56.34±1.82 and 62.22±2.30 for serum and plasma, respectively. The mean plasma concentration was higher than the mean serum concentration, but the differences were not statistically significant (P=0.06). While toxic concentrations in the liver and kidney have been reported, there are no blood data for Al in sheep and the serum reference concentration is reported for humans only. Owing to the lack of information on Al toxicity in sheep, experimental acute toxic concentration studies of blood (whole blood, plasma, and serum) are required for Al in sheep. In conclusion, no statistical difference was found between serum and plasma concentrations, and no clinical signs were observed at these concentrations.

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INTRODUCTION

Aluminum (Al) is the third most abundant element in the Earth's crust and is used in the pharmaceutical, cosmetics and food industries. It is not essential for animals and has toxic effects above tolerable concentrations (Altınok-Yipel, 2022a; Bengt, 2015; Gupta, 2012; Klein, 2019; Klotz, 2017; Lavanya, 2021; Rahimzadeh, 2022; San Martín, 2022; Uysal, 1990). Al is neurotoxic for humans and animals because it can cross the blood-brain barrier and the nervous system is the target system (Altınok-Yipel, 2022a; Bengt, 2015; Klein, 2019; Klotz, 2017; Yokel, 2000). It is considered a major risk factor for Alzheimer's and other neurodegenerative diseases (Ali and Khan, 2018; Lavanya, 2021; Yokel, 2000). It is also associated with skeletal, respiratory, immune, cardiac, and hematological disorders (Badea, 2019; Carbonara, 2023; Gupta, 2012; Klein, 2019). Most of the Al is stored in the bones and lungs, but small amounts can also be stored in the muscles and liver. It can be found in the blood (as bounded to the transferrin protein as 80-90%) and other body fluids (lymph, urine, cerebrospinal fluid, etc.) (Rahimzadeh, 2022; San Martín, 2022; Skibniewska and Skibniewski, 2019). Al is excreted by the kidneys. Normally tolerable concentrations may be toxic in patients with renal disease. It can accumulate in bones in patients on peritoneal or hemodialysis (Klein, 2019; San Martín, 2022). In addition, Al exposure in pregnant rats has been reported to increase tissue (liver, kidney, bone, and brain) concentrations of some essential trace elements (Cu, Fe, Mg, Mn and Zn), and this change may alter the level of oxidative stress (Bellés, 2001; Guo and Wang, 2011). However, a significant positive correlation has been reported between Al and body composition such as body weight, body mass index, fat-free mass, muscle and fat mass, fat percentage and internal adiposity, mineral and protein weight, body density and basal metabolic rate (Çetin, 2016).

Inhalation, dermal and oral exposure can occur from contaminated food, Al containers, and additives (Badea, 2019; Bengt, 2015; Rahimzadeh, 2022). Another important route of exposure is the administration of Al-containing vaccines (adjuvants), drugs and fluids (hemodialysis solutions and parenteral nutrition solutions) (Altınok-Yipel, 2019; Bengt, 2015; Gupta, 2012; Klein, 2019; Rahimzadeh, 2022; San Martín, 2022). Determination and monitoring of non-essential elements such as Al are clinically important because they can cause toxic effects above tolerable concentrations and can alter essential macro and microelement concentrations (Cu, Fe, Se and Zn) (Altınok-Yipel, 2022a; Altınok-Yipel, 2022b; Altınok-Yipel, 2022c; Gupta, 2012; Nordberg, 2015; Prashanth, 2015; Tiwari, 2010).

Laboratory tests that determine hematological and biochemical parameters in plasma or serum samples are important tools for clinical diagnosis in human and animal health (Akdag and Kader, 2021; Altınok-Yipel, 2022b; Kara, 2024). Absorption, accumulation, and circulation processes of elements associated with potential toxicity can be monitored in the blood. Blood and other fluids, tissues, or organs (urine, hair, liver, etc.) are often used as bioindicators to assess health status for elemental toxicity (Altınok-Yipel, 2022b). It has also been reported that sheep and some other animal species (dogs, cats, etc.) are used as indicator species to assess environmental pollution (Altınok-Yipel, 2022a).

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is a spectroscopic method of analysis used in the determination of toxic trace elements such as Al, including low concentrations at the ppt level in the blood (serum and plasma), capable of multi-element analysis, aiding precise and accurate medical diagnosis (Cedeño, 2020; Kricka and Park, 2018; Martín, 2022; Triantafillidis, 2023). It is suitable for determining trace element concentrations in small-volume samples such as blood and has become the method of choice in recent years (Komarova et al., 2021). According to the literature review, no reference concentrations have been reported for Al in sheep serum or plasma (Durak et al., 2024).

The aim of this study was to determine and compare Al concentrations in sheep serum and plasma using the ICP-MS method.

MATERIAL and METHODS

Blood Samples

As part of the study, blood samples (without anticoagulant and with heparin) were collected in tubes from the jugular vein of 12 healthy (according to clinical examine of body temperature, heart rate, respiratory rate, capillary refill time, physical condition, appetite, etc.) sheep (1-4 years old Awassi) fed and watered ad libitum under the approval of the local animal experimentation ethics committee (no: 2022/03-03), serum and plasma were then obtained by centrifugation at 4000 rpm for 10 min and stored at -80 °C.

Aluminum Analysis

The concentrations of Al in the serum and plasma samples were determined by ICP-MS under the conditions given in Table 1. The method was validated according to the parameters of limit of detection (LOD) and limit of quantification (LOQ) (μg L^{-1}), recovery (%), correlation coefficients (r^2) and relative standard deviation (%RSD) (Table 2).

Table 1

ICP-MS Operating Conditions

Plasma power	Pump speed	Plasma flow	Auxilary flow	Nebulizer flow
1300 W	20 rpm	9.0 L min^{-1}	1.65 L min^{-1}	0.93 L min^{-1}

Table 2

Validation Results of Al Analysis Method in ICP-MS

LOD (μg L^{-1})	LOQ (μg L^{-1})	Recovery (%)	r^2	RSD
2.14	8.08	93.8	0.999	1.9

Statistical analysis

After homogeneity (Levene) and normality (Shapiro-Wilk) distribution tests of the data obtained, the statistical difference between the groups was tested by independent samples t-test with SPSS software version 27.0 (IBM Corp., USA). The significance level was set at. 05 (Selvi, 2024).

RESULTS

Serum and plasma Al concentrations (μg L^{-1}) of sheep blood samples determined by ICP-MS are presented in Table 3 as the arithmetic mean, standard error, standard deviation, median, geometric mean, minimum and maximum.

Table 3

Serum (n=12), Plasma (n=12) and Total (n=24) Arithmetic Mean, Geometric Mean, Minimum and Maximum Al Concentrations (μg L^{-1})

	Serum	Plasma	*P*	Total
Arithmetic mean	56.34	62.22	0.06	59.28
Standard error	1.82	2.30		1.56
Standard deviation	6.30	7.97		7.64
Median	57.97	60.81		59.44
Geometric mean	56.01	61.74		58.80
Minimum	45.31	45.96		45.31
Maximum	66.06	75.49		75.49

Although the mean plasma Al concentration was higher than the mean serum concentration, the difference was not statistically significant.

DISCUSSION

Aluminum concentrations can be measured in tissues, hair, urine, feces and blood (Gupta, 2012). Although hair or bone analysis is the predominant means of determining Al toxic exposure, sample preparation procedures and the need for standardization should be considered (Badea, 2019; Wołowiec, 2013). The preference for blood samples in Al analysis includes clinically simpler and more applicable methods. In addition, blood samples are often used in routine clinical practice (Carbonara, 2023; Komarova, 2021; Martín, 2022). When assessing the toxicological accumulation of elements, it may be a good approach to priorities the target organ, especially in the case of low long-term exposure to Al, where the measurement of Al concentrations in the serum may not reflect accumulation in tissues (Carbonara, 2023; Van Landeghem, 1998). However, serum is useful for measuring acute exposure (Carbonara, 2023; Martín, 2022; Tomza-Marciniak, 2012). Therefore, it is considered a more accurate approach to prefer serum and plasma samples for the investigation of acute Al exposure and target tissues for chronic exposure. Carbonara, (2023) stated that the definitions of 'Al toxicity' and 'Al deposition in bone' should be distinguished (Carbonara, 2023). Al concentrations above a certain level in the diet (1200 ppm) have been suggested to be toxic to sheep (Gupta, 2012).

Although toxic concentrations of Al have been reported in the liver (6-11 ppm) and kidney (4-5 ppm) of sheep, there are insufficient data on serum and plasma concentrations (Durak, 2024; Gupta, 2012). However, as with some elements, there is insufficient information on the ideal matrix (whole blood, plasma, serum) for measuring Al blood concentrations. In the present study, although there was no statistical difference between serum and plasma, the mean plasma Al concentration was higher than the serum concentration. The higher Al concentration in plasma than in serum can be explained by the fact that most of the Al in plasma is bound to proteins (Greger, 1997; San Martín, 2022).

Durak (2024) found the mean serum Al concentration to be 2.7 ppm in a study of 313 healthy sheep (Durak, 2024). This result is higher (~45 times) than the mean concentration of our study. Although not possible to determine whether the determined mean concentration was within

physiological limits, were can within the acceptable range because the animals appeared healthy was stated by authors (Durak, 2024).

Komarova (2021) reported a plasma Al concentration of 6.9 μg L^{-1} measured by ICP-MS in a human study. (Komarova, 2021). Reference values for serum Al concentration in humans have been established <5-7 μg L^{-1} in serum, 16 μg L^{-1} in urine and <20-60 μg L^{-1} in dialysis patients. (Bengt, 2015; Martín, 2022). The critical concentration at which neurological symptoms occur in humans is 100 μg L^{-1} in urine. (Rahimzadeh, 2022). No toxic reference concentration for Al in sheep has been reported. Serum Al concentrations are lower than those in other tissues (Greger, 1997).

There is insufficient information on the acute toxic effect of Al in ruminants leading to mortality (Thompson, 1991). Studies have shown that some of the orally ingested Al is excreted in the urine, whereas some accumulates in the tissues. This accumulation varies depending on various factors (age, disease, kidney function, etc.) (Greger, 1997). When studies on species-specific toxic Al blood concentrations are reviewed, studies on sheep are quite insufficient (Durak, 2024).

In toxicity studies of Al (neurotoxicity, embryotoxicity, etc.) in mice, rats, and dogs, the lowest concentrations of adverse effects were 52, 75, and 100 mg kg^{-1} BW day^{-1} respectively. Acute oral toxicity concentrations for Al compounds in mice and rats (mouse LD_{50}: 164- >730; rat LD_{50}: 162- >730) have been reported over a wide range (Aguilar, 2008).

Al accumulation may be observed in patients with chronic kidney disease or hemodialysis patients (dialysis solutions containing Al). It has been reported that blood Al concentrations in hemodialysis patients should not exceed 20 μg L^{-1}. Monitoring of Al in at-risk dialysis patients recommended (Martín, 2022).

CONCLUSION

Al is an important toxic element that has adverse effects (especially neurological) on many systems and organs. It has a potential for exposure to humans and animals through environmental and food contamination. Also, Al toxicity has been observed in some diseases such as renal diseases. However, there is a lack of information on Al toxicity in animal species. In particular, studies on blood concentrations and reference ranges of Al in sheep are inadequate. Furthermore, the choice of sample (whole blood, serum, plasma) according to the toxic dynamic and kinetic properties of the elements (binding to proteins, etc.) is essential for the accurate determination of blood concentrations, which play an important role in toxic element analysis.

In conclusion, no statistical difference was found between serum and plasma concentrations in terms of sample prefered for Al analysis. In addition, no clinical signs of toxicosis or diseases were observed at the determined plasma and serum concentrations. Therefore, experimental acute toxic concentration studies in sheep, including whole blood, plasma and serum concentrations are required.

Ethics Committee Approval

22/03/2022 dated and 2022/03-03 numbered was given by Hatay Mustafa Kemal University, local animal experimentation ethics committee.

Author Contributions

All authors contributed to the study conception and design.; Methodology: Fulya ALTINOK-YİPEL; Sampling and laboratory analysis: Fulya ALTINOK-YİPEL, Mustafa YİPEL Statistical

analysis and interpretation of the data: Mustafa YİPEL; Drafting: Fulya ALTINOK-YİPEL; all authors commented on previous versions of the manuscript; Reviewing and editing: Fulya ALTINOK-YİPEL, Mustafa YİPEL.

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Conflict of Interest

Please indicate whether there is a conflict of interest.

Sustainable Development Goals (SDG): 3 Good Health and Well-Being

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Determination of the Effect of Melatonin and Vitamin E on Cadmium Chloride-Induced Pathologies in the Stomach and Small Intestine of Lohmann Chicken Embryos

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ABSTRACT

Cadmium (Cd), one of the most important heavy metals contributing to environmental pollution, represents a significant risk factor for public health. The human body is exposed to cadmium through the ingestion of food and respiration. Cadmium accumulates in tissues such as the stomach and small intestine, where it can cause pathological changes in the organs. Melatonin is an antioxidant hormone secreted by the pineal gland, whereas vitamin E is a powerful antioxidant with therapeutic properties. The aim of this study was to determine the toxicity of Cadmium chloride ($CdCl_2$) in the stomach and small intestine of Lohmann breed chicken embryos and to treat them with melatonin and vitamin E.A total of 30 fertile eggs of the Lohmann breed were utilized in this study, and five experimental groups were established (n=6): distilled water (DW), DW+ ($CdCl_2$), DW+Melatonin, DW+Vitamin E, DW+$CdCl_2$+Melatonin+Vitamin E. After a single dose of $CdCl_2$ (0.430 mM), Melatonin (5 mg) and vitamin E (3 mg) were administered to each egg via in-ovo injection, the hole was closed with liquid paraffin. The fertile eggs were transferred to the incubator and the study was completed before the chicks hatched (day 21). The stomach and small intestine tissue of the chicks were excised, fixed in 10% formaldehyde, and processed using an alcohol and xylene series. Following the completion of routine histological procedures, the samples were stained with Hematoxylin-Eosin and examined under a light microscope. A decrease in average body and organ weight was observed in the $CdCl_2$ group, while the mixture of melatonin and vitamin E prevented this decrease. The administration of $CdCl_2$resulted in the necrosis and hemorrhage of gastric epithelial cells, as well as the deformation of small intestine crypts, the rupture and fracture of villi, and hemorrhage. The administration of $CdCl_2$ induced pathologies in the gastrointestinal system, and the mixture of Melatonin and Vitamin E may have a limited effect in treating the damage. Further studies are required to determine the protective properties of melatonin and Vitamin E against $CdCl_2$ in different experimental animals.

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INTRODUCTION

Nowadays, increasing industrial activities and the release of heavy metals into the environment due to natural phenomena represent significant challenges that pose a threat to public health. Heavy metals exhibit a high degree of resistance to adverse environmental conditions, which enables them to persist in nature and in the human body for extended periods (Şavran and Küçük, 2022). Cadmium (Cd) is a heavy metal with which humans, at the top of the food pyramid, are in constant contact (Blasiak, 2001). Cd is a white element with an atomic number of 48, a density of 8.64 g ml, an atomic weight of 112.4, and a valency of "+2" (IPCS, 2005; IPCS, 2007). In its natural state, Cd is found in a variety of forms, including Cd oxide, chloride, sulfide, and sulfate. It can be ingested orally through food or inhaled in powder or aerosol form. It has been reported that Cd, which is known to have toxic effects in both forms (Yıldızgören et al., 2014), causes pathology by accumulating especially in the stomach and small intestine, as well as in the lungs, liver, kidneys, pancreas, and prostate (Demirkıran et al., 2020). The half-life of Cd is approximately 30 years (Coşan et al., 2017), it reduces vitamin D levels, and it negatively affects bone mineralization (James and Meliker, 2013). Advanced pathologies have been documented in individuals engaged in occupational activities with high exposure to Cd oxide compounds (Ateş, 2008).

Sources of Cd, such as foods and contaminated drinking water, as well as industrial activities like battery production, textiles, electronics, and refineries (Coşan et al., 2017; Rani et al., 2014), may also negatively affect the endocrine system. Cd causes an increase in oxidative stress by disrupting the functioning of natural enzyme groups such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px), which are known as antioxidants and are involved in reducing oxidative stress (Cuypers et al., 2010; Güneş and Şensoy, 2022). The World Food and Agriculture Organization (FAO) and the World Health Organization (WHO) have established the acceptable value of Cd for humans (Acceptable Daily Intake; ADI) for nutrients at 5 ppm/kg/day and for drinking water at 0.005 mg L day (Kara et al., 2016). Some therapeutic agents may play an active role in the treatment of pathologies caused by Cd toxicity and in the reduction of oxidative stress. Melatonin, a neurohormone secreted from the pineal gland, small intestine, skin, and leukocytes, is a natural antioxidant with therapeutic properties (Baltacı 2001; Baran et al., 2020; Karaca et al., 2014; Öner et al., 2003). Melatonin plays an active role in ensuring sleep patterns and coordinating the immune and reproductive systems (Candan et al., 2017). Furthermore, it plays an active role in preventing oxidative damage (Forsling, 2001) because of its ability to trap free oxygen radicals, prevent lipid peroxidation, and activate SOD, GSH-Px, and CAT enzymes (Erdem 2010; Özmete 2009; Uluocak et al., 2010). Vitamin E is a powerful antioxidant with therapeutic properties that can suppress free oxygen radicals (Pranay Kumar et al., 2019).

The easiest and cheapest method to determine the Cd toxicity process is the use of live animals. This method not only permits the active ingredient to be tested in animal tissues but also offers a significant advantage in terms of time. In studies on the therapeutic aspects of melatonin and vitamin E, a variety of experimental animals have been used (El-Sokkary et al., 2010; Gültekin et al., 2001; Kim et al., 1998; Karaca et al., 2014). Poultry holds an important place in histopathological studies due to its short incubation period, high reproductive capacity, ease of breeding, low rearing costs, and genetic diversity. Chicken embryos were preferred to determine the effects of melatonin and vitamin E against Cd damage because they have physiological systems that are similar to those of humans and their histological structures are largely similar (Shabalout et al 2023). In the literature, studies investigating the efficacy of natural therapeutic agents in the treatment of $CdCl_2$ toxicity in fertile eggs. The results of these studies are often contradictory. The aim of this study was to treat pathologies induced by $CdCl_2$ toxicity in the stomach and small intestine of Lohmann breed chicken embryos with melatonin and vitamin E.

MATERIAL METHOD

Ethics Committee Permission

The ethics committee permission was obtained from the Selcuk University Faculty of Veterinary Medicine Experimental Animal Production and Research Center Ethics Committee (16.02.2021, 2021/01-18).

Material

Lohmann breed fertile eggs were used (n: 30). The eggs were examined under a light box to ensure normal porosity and disinfected with a mixture of 21 g potassium permanganate and 42 ml formaldehyde/m^3 in a steam environment for 15 min, after which they were placed in the incubator. The weights of the eggs were recorded before and after incubation.

Experimental Groups

Five groups were created (n: 6): Group I: Distilled water (DW), Group II: DW+cadmium chloride ($CdCl_2$), Group III: DW+melatonin, Group IV: DW+vitamin E, and Group V: DW+$CdCl_2$+Melatonin+Vitamin-E (Table1).

Incubation Process

The fertile eggs were maintained at 37.5±0.5°C and 65-70% humidity for 2 days to prevent heat stress before being transferred to the incubator. Throughout the incubation process, the eggs were rotated once every hour and provided with a suitable growth and living environment (maintaining a temperature of 37.8°C and relative humidity of 65%).

Table 1

Groups and Applied Procedures (n:6)

Experimental Groups	Chemical Substance	Dose
Group I	DW	20µl
Group II	DW	20µl
	$CdCl_2$	0.430 mM
Group III	DW	20µl
	Mel	5µg
Group IV	DW	20µl
	Vit-E	3µg
Group V	DW	20µl
	$CdCl_2$	0.430 mM
	Mel	5µg
	Vit-E	3µg

Applications

The level of $CdCl_2$ was determined on the basis of the human physiological dose (30 mg kg) and applied as 0.430 mM, according to literature knowledge (Nordberg et al., 2007; Venter et al., 2015). Melatonin's levels (5 µg) and vitamin E (3 µg) were also measured. Before injection, the eggs were disinfected using 70% ethanol. All applications were administered as a single dose on the eighth day of the incubation process, which is a time of intensive metabolic activation. Following the

injections, the holes were closed by applying liquid paraffin (Özparlak 2015; Wolf and Leupke 1997).

Tissue Samples

On the 21st day of the incubation process, the eggs were hatched from the blunt end. The embryos were then placed in moistened cotton jars. Once their inactivity was confirmed, the sacrifice process began. The stomach and small intestine tissues were removed, weighed, and transferred to a 10% phosphate-buffered formal-saline solution (0.1M PBS, pH: 7.4).

Histopathological Examinations

Following fixation, standard follow-up procedures were conducted. Six-micrometer (µm) sections were obtained from the paraffin-embedded tissues. Subsequently, the preparations were stained with Hematoxylin-Eosin (HE) and examined under a light microscope, with important regions being recorded (Şensoy and Öznurlu 2019).

Statistical analyzes

Experiments were conducted in 4 repetitions to give mean and standard errors. Data transferred to the SPSS package program (version 27.0, IBM Corporation, Armonk, NY) were analyzed using ANOVA and LSD tests. Analysis of variance was applied to mean body weight, mean stomach weight, and mean small intestine weight followed by LSD test for the parameters were performed with one-way ANOVA. The significance level was determined as $p<0.05$ in all statistical tests in the study.

RESULTS

Average body and organ weights

The mean body weight decreased in Group II; moreover, it did not change in Groups III and IV. We observed a decrease in Group V compared with the control, but it increased significantly compared with Group II ($p<0.05$). The mean stomach and small intestine weights decreased in Groups II and V compared with the control group ($p<0.05$), (Table 2).

Table 2

Average Body and Organ Weights of the Embryos (Mean ± SE)

Group	Mean Body Weight (g)	Mean Stomach Weight (g)	Mean Small Intestine Weight (g)
Group I	38.25±2.03[a]	1.09±0.01[a]	4.13±0.03[a]
Group II	32.12±3.44[b]	0.78±0.02[b]	3.66±0.02[b]
Group III	39.89±1.23[c]	1.05±0.01[c]	4.51±0.01[c]
Group IV	38.71±2.99[c]	1.03±0.02[c]	4.08±0.01[c]
Group V	34.63±1.37[d]	0.89±0.04[d]	3.69±0.03[d]
Sig	0.01	0.03	0.02

SE: Represents Standard Error. Values Shown With Different Letters Differ From Each Other At The P<0.05 Level.

Histopathological evaluation

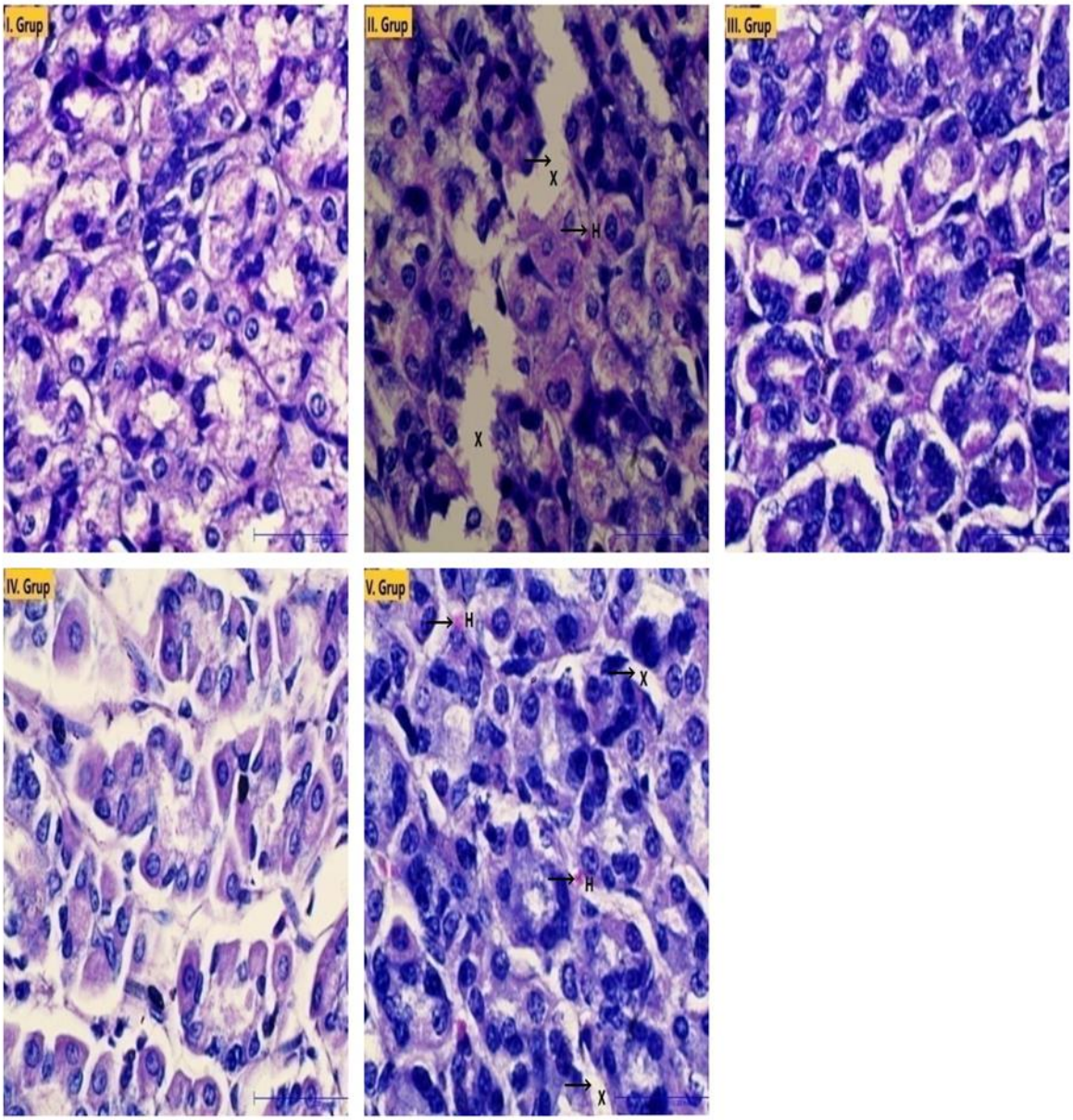
Stomach tissue

The histopathological appearance of the stomach tissue was normal in the control group,

Group III, and Group IV. In these groups, the stomach contours were regular, and the cytoplasm and nucleus were not stained darkly. Additionally, there were no gaps or hemorrhages between the epithelial cells. In Group II, the nucleus and cytoplasm were stained dark, and gaps and hemorrhage were observed between the epithelial cells. In Group V, the nucleus and cytoplasm stained normally, but there were partial gaps and hemorrhage between the epithelial cells (Figure 1).

Figure 1

Light Microscopy Images of Stomach Tissue (HE Staining, Magnification:100 µm)
Spaces Between Epithelial Cells: X, Hemorrhage: H).

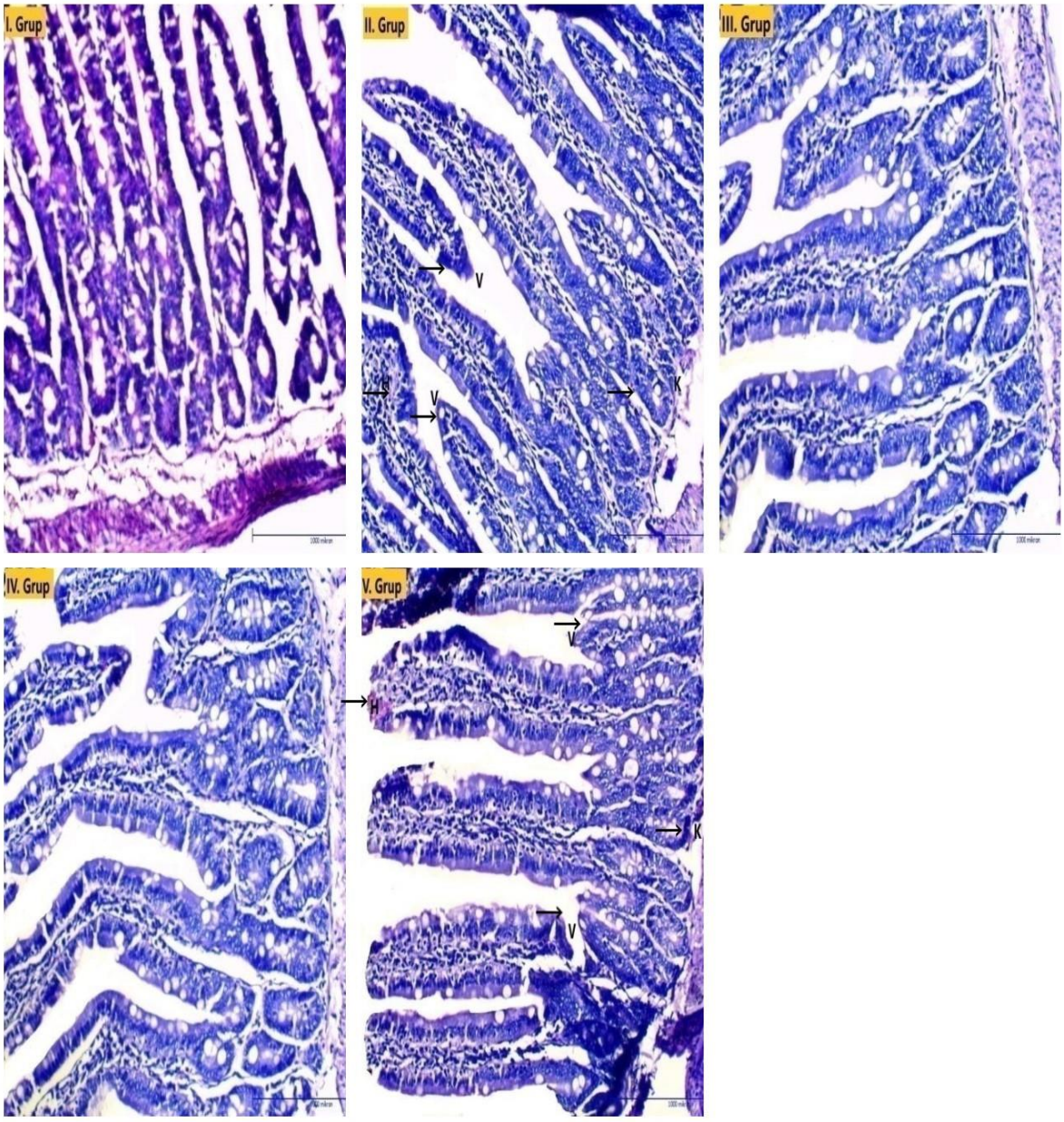


Small intestine tissue

The histological structure of the small intestine tissue in the control group, Group III, and Group IV was normal, and the contours of the mucosal layer were smooth. In these groups, no deformations in the crypts, ruptures, fractures, necrotic areas, or hemorrhage were observed. In Group II, deformations in the crypts, ruptures, fractures, and extensive hemorrhage were detected in the villi. In Group V, deformations of the crypts, rupture of the villi, and hemorrhage were detected (Figure 2).

Figure 2

Light Microscopy Images of Small Intestine Tissue (HE Staining, Magnification: 100 µm, Deformation In The Crypts: K, Ruptures And Fractures In The Villi: V, Hemorrhage: H).



DISCUSSION

The accumulation of heavy metals in the environment is a growing concern, as it has the potential to negatively impact public health. Heavy metals, which have very long half-lives, accumulate in tissues and organs, causing pathologies. Heavy metals such as Cd cause developmental disorders in organs and a reduction in average body and organ weights, which can be attributed to alterations in the protein, carbohydrate, and fat balance of cells (Şensoy 2023). Because the embryonic period is when growth and development are the fastest, Cd may cause retardation in organ development (Çağlar and Saral 2014). The impact of Cd during the embryonic period has been investigated by numerous researchers on different experimental animals, and contradictory results. For instance, in one study, a single dose of $CdCl_2$ (5 µg) and $Pb(NO_3)_2$ (50 µg) was administered to fertile chicken eggs by in-ovo injection, resulting in a reduction in average body weight (Kril et al., 2011).

Another study found that fertile Lohmann breed eggs administered three doses of Cd (50.2 mg kg) by in-ovo injection led to increased relative intestinal weights and decreased body weights (Berzina et al., 2007). In contrast, a different study reported a decrease in body weight in 100-day-old Ross breed chickens fed $CdCl_2$ (50 and 100 ppm) in their diets (Teshfam et al., 2006). Similarly, a study reported that six-week-old chickens fed Cd (10 mg day) for four weeks experienced a decrease in body weight (Ali et al., 2016). In our study, the average body, stomach, and small intestine weights were affected by $CdCl_2$, with our results indicating a decrease in weight that is consistent with similar studies. It is thought that the observed decrease in average weight values is due to alterations in the carbohydrate, protein, and fat composition of tissues and organs resulting from $CdCl_2$ exposure.

Cd toxicity can directly or indirectly affect all systems. The gastrointestinal system is one of the first systems to be affected, as Cd is mostly ingested through food (Anetor 2012). Short-term Cd exposure affects the liver, while long-term exposure affects the intestines, stomach, kidneys and heart (Valko et al., 2005). Cd is absorbed from the small intestine, where it accumulates in the intestinal mucosa with little diffusion into the organism (Bishak et al., 2015). Cd accumulation in the proximal region of the human intestine causes degeneration and severe gastrointestinal disorders (Şimşek and Alabay 1999). Cd toxicity leads to increased lipopolysaccharide production and changes in metabolic activity, triggering cellular damage in the intestinal wall (Tinkov et al., 2018). In a study, 100-day-old Ross breed chickens fed $CdCl_2$ (5, 50, and 100 ppm) for 49 days showed decreased villus length, width and crypt depth (Teshfam et al., 2006). Berzina et al. (2007) reported that Lohmann breed brown roosters orally fed Cd (50.2 mg kg) for 3 days showed decreased villus length. In addition, it was Cd decreased the rate of Ca^{+2} absorption in the intestine of chicks (Fulmer et al., 1980). In a similar study, Cd showed radioactivity in various parts of the small intestine of newborn rats and was retained in the intestine for a long time (Sasser and Jarboe, 1977). Cd also increases metallothionein expression (Danielson et al., 1982) and causes damage and inflammation in the small intestine of mice (Ninkov et al., 2015). In addition, Cd-induced intestinal damage causes irregularities in the microbiota, which leads to a decrease in body resistance (Velayatzadeh 2023). However, some studies have reported intestinal resistance to heavy metals, with no signs of atrophy, necrosis or hyperplasia in the small intestinal mucosa of Cd-fed rats (Nai et al., 2013).In various models of Cd toxicity, it has been shown to cause nuclear degeneration (Younis et al., 2016), inflammation (Liu et al., 2019), villi erosion, necrosis, vacuole formation, and irregularities in the lamina propria of the intestinal mucosa (Duan et al., 2023; Li et al., 2017; Xie et al., 2019; Yu et al., 2021).

Because the gastric mucosa is very sensitive to Cd, in cases of toxicity, the protective structures of the gastric mucosa weaken and become more susceptible to stress (Öner et al., 1994). Rats orally administered $CdCl_2$ (15 ppm/day) for 30 days experienced decreased stomach acid and mucin values, dilated mucosal vessels, decreased parietal cell numbers, and tubule vesicular membrane ruptures (Asar et al., 2002). In addition, cellular degeneration, necrosis, and vesicle formation in gastric tissue were observed. Similarly, mild dysplasia and interstitial inflammation have been detected in the gastric mucosa of rats fed $CdCl_2$ via water (Nai et al., 2013). Our results, which indicate that Cd causes necrosis and hemorrhage among epithelial cells in stomach tissue and deformation of the crypts, ruptures and fractures, and hemorrhage in the villi of the small intestine, are consistent with similar studies.

There is no effective treatment for cadmium toxicity. Studies in the literature have used agents with therapeutic effects in various models (El-Sokkary et al., 2010; Karaca et al., 2014; Kim et al., 1998). It has been reported that melatonin (single dose 10 mg kg), a hormone with therapeutic effects, reduces Cd-induced inflammation, regulates cytokine expression and accelerates proliferation in fertile chicken eggs (Li et al., 2018). Similarly, melatonin was reported to increase enzyme levels in rats whose antioxidant enzyme levels were decreased by CdCl2 toxicity (Aydoğdu et al., 2007). Vitamin

E, a therapeutic and strong antioxidant, reduces reactive oxygen molecules generated by aerobic respiration and increases antioxidant defensive capacity (Pranay Kumar et al., 2019). It has been reported that fertile eggs administered vitamin E (30 mg) by novo injection showed reduced mortality and increased immunity levels (Salary et al., 2014). According to Goel et al. (2013), vitamin E increases IgM levels in chicks. There are no studies in the literature combining melatonin and vitamin E for the treatment of cadmium toxicity. The results of our study, which are expected to shed light on future studies in this area, indicate that the combination of melatonin and vitamin E has a limited therapeutic effect in treating $CdCl_2$ toxicity. The combination of melatonin and vitamin E, which prevents the decrease in average body and organ weights due to $CdCl_2$, has a limited effect in the treatment of gastric and small intestinal pathologies.

CONCLUSION

Natural therapeutic agents may indeed hold promise in mitigating the effects of CdCl2 toxicity, which affects organ development and induces pathologies. The identification of pathologies and negative developmental effects in fertile eggs exposed to $CdCl_2$highlights the importance of investigating interventions such as melatonin and vitamin E. Although these treatments showed partial efficacy, their potential warrants further investigation. Raising public awareness of Cd toxicity and advocating for measures to prevent heavy metal exposure, especially during critical developmental periods, are critical steps in protecting public health. By promoting awareness and advocating for preventive measures, we can minimize the adverse effects of heavy metal exposure on human health and development.

Ethics Committee Approval

16/02/2021 dated and 2021/01-18 numbered was given by Selcuk University Faculty of Veterinary Medicine Experimental Animal Production and Research Center Ethics Committee.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by EŞ and EG. The first draft of the manuscript was written by EŞ and EG, with input and revisions from other authors. All authors have read and approved the final manuscript.

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Conflict of Interest

The author declares that there are no conflicts of interest.

Sustainable Development Goals (SDG): 3 Good Health and Well-Being

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Investigation of the Effect of Organic Acid Addition to White Layer Akbay Line Chickens' Water on Villi and Some Egg Quality Parameters

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ABSTRACT

Organic acids are carboxylic acids with the R-COOH structure and are released naturally through metabolic events. Organic acids are widely used as preservatives in poultry feed, especially to keep mold and other microorganisms under control. Organic acids are grouped according to the number of carbon atoms and carboxyl groups in their structures. Purpose of this study was to investigate the effects of increasing amounts of organic acid addition (Lactic acid, Formic acid, Propionic acid and Acetic acid) to laying hen rations on some parameters of egg quality, productivity and intestinal villi. In the study, 180 Akbay white laying hens at the age of 40 weeks were distributed to 3 groups and 6 replicates. Ad libitum drinking water was added to CN group chickens, 1 L/1000 L and 2 L/1000 L organic acid (85% Formic acid, Propionic acid, 80% Lactic Acid, 80% Acetic Acid) was added to the water of OAM-1 and OAM-2 group chickens and it was given 30 days. There was no statistically significant difference in the length measurement of intestinal duodenum, jejunum and ileum villi between the control and the groups to which organic acid was added ($P>0.05$). There was no lesion in the histopathological examination of the intestines. Egg productivity parameters such as egg weight, yolk color index, albumen height, Haugh unit and strength were found to be statistically significant between the groups ($P<0.05$). As a result, it is thought that it would be appropriate to add organic acid to the drinking water of laying hens for shell quality and quality egg production.

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INTRODUCTION

In poultry and wild birds, it is important to reveal histopathological changes and closely examine the intestines for the continuity of a balanced ecosystem (Kanat, 2024). Chronic or acute inflammations occurring in the small intestine mucosa can cause destruction of the villi (Yıldırım E., 2020). Increasing intestinal villi and improving egg quality are of great importance for commercial enterprises. Apart from organic acid compounds, there are also studies on yeast additions to diets (Gül et al., 2013). In commercial poultry farms, different feed additives have been used to prevent the colonization of intestinal pathogenic microorganisms, support the immune system and increase their performance (Islam et al., 2008). One of the feed additives used is organic acids.

The chemical structures of compounds called organic acids and carboxylic acids depend on the carbon skeleton (Kum and Güçlü 2006). It has been reported that there are nearly sixty classes of organic acids naturally obtained from animals, plants and microorganisms. Some of these are lactic acid, formic acid, citric acid, fumaric acid (Park et al., 2009). Organic acids (OAs), a suitable alternative to antibiotics, have positive effects on intestinal health and bird performance (Adams, 1999; Rathnayake et al. 2021; Sak and Soykut, 2021). In addition, organic acids prevent the formation of mol d and mycotoxins (Nir and Şenköylü, 2000).

Organic acids, which are added to feed or drinking water, have an antibacterial effect with their insoluble parts (İpçak et al., 2017). Organic acids lower gastric pH, rapidly convert pepsinogen into pepsin, stimulating the absorption of amino acids, proteins and minerals. (Park et al. 2009; Yeşilbağ et al. 2007). Studies have shown that the addition of organic acids to diets causes significant changes in the small intestinal microflora (Bozkurt and Sandıkçı, 2009; Gül, 2017; Yeşilbağ et al. 2007). They do not leave residue in the body after being metabolized in the organism.

Due to the expansion of the small intestine villus surface, there is an increase in absorption and utilization of nutrients. (Kara et al., 2013). Egg quality is important not only for consumer preferences but also for producers as economic gain (Akkuş and Yıldırım, 2018). In-house management is among the factors that determine quality. Egg shell quality and durability are also important in terms of hatching use, marketing, and nutritional values. (Akkuş and Yıldırım, 2018).

The purpose of this study is to investigate the effect of organic acid addition to the drinking water of white laying hen Akbay line on intestinal villus length and egg quality.

MATERIAL AND METHOD

Animal

Ethical permission was obtained from the Local Ethics Committee of Poultry Research Institute Directorate (TAE-HADYEK) (approval no: 2023/02). The animal material of the study consisted of 180 40-week-old white layer Akbay chickens. The composition of the commercial organic acid (MOLDCID LIQUID, VİMAR®, Türkiye) used in the experiment is Formic acid (85%), Propionic acid, Lactic Acid (80%) and Acetic Acid (80%). Feed was given to the chickens as ad libitum. Chickens were randomly placed into 3 groups and 6 replicates in a 3-layer battery type cage system.

Experimental Study

***Control group (CN)*:** Drinking water without organic acids was given ad libitum.

Organic Acid-1 group (OAM-1): 1 L/1000 L organic acid was added to drinking water and given ad libitum.

Organic Acid-2 group (OAM-2): 2 L/1000 L organic acid was added to drinking water and administered ad libitum.

The study was completed on the 30th day. The jugular vein of 6 of 60 chickens in each group (1 from each replicate) was cut and necropsy was performed. Intestinal sections (duodenum, jejunum, ileum) were taken into 10% buffered formalin for histopathological examination.

Histopathological Examination

Necropsies were performed on the chickens, and the tissues were kept in buffered 10% formalin for 48 hours. The tissues were kept under running tap water for 8 hours to remove formalin residues. The tissues were trimmed and transferred to tissue tracking cassettes. The tissue tracing process was passed through ascending alcohol series, xylene series and paraffin series and blocked with paraffin. 5 µm thick sections were taken from the obtained blocks. Sections were stained with the Hematoxylin-Eosin staining method (Luna, 1968). Sections were examined under a light microscope (Leica DM2500). Length measurements of the villi were made with the Leica Application Suite V4.12.0 program.

Determination of Egg Quality Parameters

At the beginning and end of the experiment, internal and external quality characteristics (egg weight, breaking strength and shell thickness, albumin height, yolk color index and haugh unit) of 20 eggs randomly selected from each group were determined using the DET-6000-Digital Egg Tester measuring device (Nabel).

Statistical Analysis

Data were analyzed using software package Minitab version 19. All values are presented as Mean ± Standard Error (SE). To test the significance of the difference in group comparisons, analysis of variance was performed in repeated measurements in independent groups at the 5% significance level. The assumption of homogeneity of variances was examined with the sphericity test. Intestinal villus length and egg quality parameters were statistically analyzed by one-way analysis of variance (ANOVA) between study groups. Significant differences were determined after the data were analyzed by Tukey's multiple range test. $P<0.05$ was considered to be statistically significant.

RESULTS

Histopathological Results

The villus lengths of the intestinal sections from the chickens of the control and treatment groups are given in Table 1. No statistically significant difference was found between the intestinal duodenum, jejunum and ileum villus lengths of the study group chickens to which organic acid was added to their water ($P>0,05$, Fig 1 A-B-C). No pathological findings were found in the histopathological examinations performed on the intestinal sections.

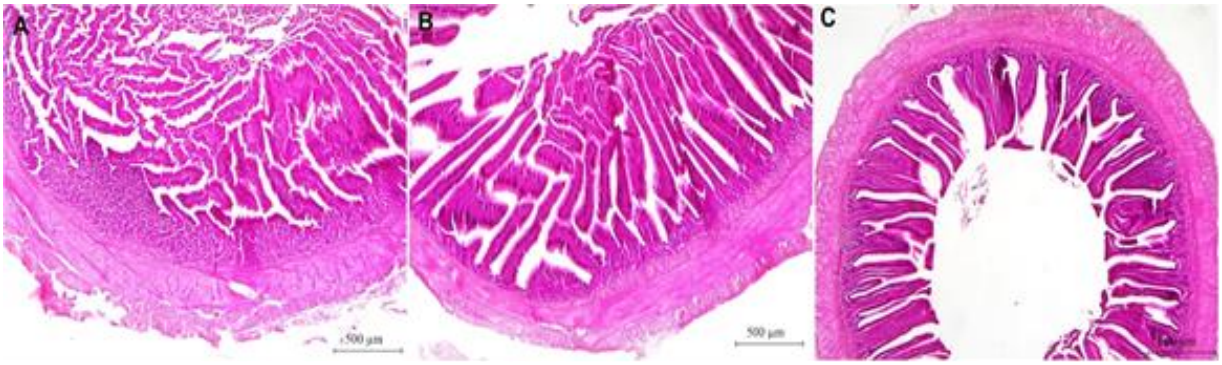
Table 1

Statistically Significant Differences in the Effect of Organic Acid Added to Drinking Water of White Layer Akbay Line Chickens on the Length of the Intestinal Duodenum, Jejunum and Ileum Villus

Variable	Group	N	N*	Mean± SE	Minimum	Maximum	*P value*
Duodenum	CN	6	0	1436.0±57.6	1245.0	1595.0	0.171
	OAM-1	6	0	1352.5±27.4	1262.0	1431.0	
	OAM-2	6	0	1479.3±46.8	1374.0	1685.0	
Jejenum	CN	6	0	1040.8±64.4	879.0	1306.0	0.917
	OAM-1	6	0	1058.3±62.1	891.0	1254.0	
	OAM-2	6	0	1081.7±80.2	770.0	1323.0	
Ileum	CN	6	0	852.7±59.5	651.0	1028.0	0.769
	OAM-1	6	0	906.3±92.0	644.0	1178.0	
	OAM-2	6	0	918.5±42.4	790.0	1068.0	

Figure 1

Normal Histological Appearance of the Intestine. White Layer Akbay Line Chicken.
A. Duodenum. Oam-2 Group. H&E. Bar:500 µm. B. Jejunum. Oam-1group. H&E.Bar:500 µm. C. Ileum. Oam-2 Group. H&E.Bar:500 µm.



Egg Quality Parameters

Findings regarding egg quality characteristics are given in Table 2. At the beginning of the study, the yellow color index of eggs taken from chickens was lowest in the OAM-2 group and highest in the OAM-1 group (P<0.05). Additionally, there was no statistically significant difference between the groups in egg weight, shell thickness and strength and also Haugh unit (P>0.05).

At the end of the experiment, egg weight and strength, yolk color index, Haugh unit, albumen height parameters were found to be statistically significant between control and treatment groups (P<0.05). Egg weight and yolk color index were higher in the OAM-1 group, albumen height and Haugh unit were higher in the control group, and strength value was higher in the OAM-1 and OAM-2 groups (P<0.05). There was no statistically significant difference in eggshell thickness between the control and treatment groups (P>0.05).

DISCUSSION

Three important elements of the gastrointestinal tract are the immune system, intestinal microbiota and epithelial cells. The main part of the intestine where the absorption of nutrients takes place is the small intestinal villi. Prebiotics, such as organic acids and essential oils, improve the

integrity of intestinal epithelial cells, additionally enhancing the absorption of molecules and improving the growth performance of productivity animals (Adil et al. 2010; Kaya et al. 2015). Organic acids like fumaric, citric, propionic and formic acids increase gastric proteolysis, significantly affecting the digestibility of proteins and amino acids. (Samanta, 2010). In this study, the effect of organic acid added to the drinking water of white layer Akbay chickens on intestinal villus length and egg quality was investigated.

Table 2

Statistically Significant Differences in the Effect of Organic Acid Added to the Drink Water of White Layer Akbay Line Chickens on the Egg Quality Characteristics.

	Variables	Group	N	Mean±SE	*P value*
Beginning of study	Egg Weight	CN	20	59.54± 0.59	0.160
		OAM-1	20	61.13± 0.90	
		OAM-2	20	61.88± 1.05	
	Albumen Height	CN	20	7.535± 0.14	0.264
		OAM-1	20	7.745±0.19	
		OAM-2	20	7.260± 0.27	
	Yolk Color Index	CN	20	11.245± 0.15 [ab]	0.020*
		OAM-1	20	11.680± 0.16 [a]	
		OAM-2	20	11.115± 0.11 [b]	
	Haugh Unit	CN	20	86.925± 0.80	0.119
		OAM-1	20	87.63± 1.01	
		OAM-2	20	84.15± 1.71	
	Strength	CN	20	44.97± 1.72	0.255
		OAM-1	20	44.65± 1.23	
		OAM-2	20	41.85± 1.36	
	Eggshell Thickness	CN	20	0.345± 0.00	0.376
		OAM-1	20	2.52± 2.18	
		OAM-2	20	0.344± 0.00	
End of study	Egg Weight	CN	20	59.420± 0.59 [b]	0.050*
		OAM-1	20	61.895± 0.83 [a]	
		OAM-2	20	60.155± 0.70 [ab]	
	Albumen Height	CN	20	7.450± 0.20 [a]	0.000*
		OAM-1	20	7.115± 0.58 [a]	
		OAM-2	20	4.940± 0.14 [b]	
	Yolk Color Index	CN	20	11.370± 0.08 [ab]	0.005*
		OAM-1	20	11.720± 0.15 [a]	
		OAM-2	20	11.045± 0.17 [b]	
	Haugh Unit	CN	20	86.33± 1.12 [a]	0.000*
		OAM-1	20	82.17± 2.44 [a]	
		OAM-2	20	67.73± 1.34 [b]	
	Strength	CN	20	37.79± 2.27 [b]	0.012*
		OAM-1	20	41.44± 1.94 [a]	
		OAM-2	20	46.69± 1.91 [a]	
	Eggshell Thickness	CN	20	0.336± 0.00	0.198
		OAM-1	20	0.345± 0.00	
		OAM-2	20	0.347± 0.00	

*:P<0,05

Organic acids control the proliferation of many pathogenic and non-pathogenic intestinal bacteria. It also increases the height of villi and the secretion, digestion and absorption of nutrients by the mucosa by reducing inflammatory reactions in the intestinal mucosa (Adil et al. 2010; Samanta et al. 2010). In studies investigating organic acid on intestinal villus length in chickens, Samanta et al. (2010) reported that the villus height in the duodenum increased linearly with the dose of organic acid mixture. Found that organic acid dietary supplementation in broiler chickens significantly increased duodenum and jejunum villus length ($P<0.05$) compared to the control group. In this study, no statistically significant difference was found between the intestinal duodenum, jejunum and ileum villus lengths of the study group chickens to which organic acid was added to their water ($P>0.05$).

Eggs are foods that contains a high amount of animal protein and is cheap and easy to produce. In recent years, with the increase in human population, the demand for egg production has also increased (Ürüşan and Bölükbaşı, 2020). In addition, eggs increase antioxidant activity and phenolic substance content in foods offered for human consumption (Kömürcü and Bilgiçli, 2024).

It is important for eggs, which are important as a source of protein for humans, to have a strong shell. In laying hen farms, 8-10% of eggs break due to shell problems and this causes economic losses (Tabib and Onbaşılar, 2019). Egg shell thickness and breaking resistance, egg Haugh unit, yolk color index and albumen height are affected by feed and feed additives added to rations and drinking water (Ürüşan and Bölükbaşı, 2020). Organic acids maintain electrolyte balance by enabling anions to form compounds with minerals such as Ca, Mg, Zn and P in chicken diets and intestines (Kaya et al., 2014). In this study, it was determined that in the groups where organic acid was added to the water of chickens, egg weight and yellow color index increased in the OAM-1 group, and the strength value increased in the OAM-1 and OAM-2 groups ($P<0.05$). Kaya et al. (2020) added 0, 1.5, 3 and 4.5 kg/ton of organic acid to chicken rations for 13 weeks. It was determined that the rate of damaged eggs decreased and egg weight increased in the groups to which organic acid was added. Dama and Kaya (2018) reported in their study that adding propionic acid at increasing levels (0.100, 200 and 300 ppm) to laying hen rations caused changes in egg quality parameters (shell thickness and breaking strength, egg yellow color index, Haugh unit and albumen height). In this study, it is thought that adding 1 L/1000 L organic acid supplement to the water of chickens is more effective on egg quality.

In the conclusion, in our study, it was determined that the addition of organic acid to the drinking water of laying hens was effective on egg weight, shell breaking resistance, shell thickness and yellow color index. We think that it would be appropriate to add organic acid to the drinking water of laying hens for shell quality and profitable egg production.

Three important elements of the gastrointestinal tract are the immune system, intestinal microbiota and epithelial cells. The main part of the intestine where the absorption of nutrients takes place is the small intestinal villi. Prebiotics, such as organic acids and essential oils, improve the integrity of intestinal epithelial cells, additionally enhancing the absorption of molecules and improving the growth performance of productivity animals (Adil et al., 2010; Kaya et al., 2015). Organic acids like fumaric, citric, propionic and formic acids increase gastric proteolysis, significantly affecting the digestibility of proteins and amino acids. (Samanta, 2010). In this study, the effect of organic acid added to the drinking water of white layer Akbay chickens on intestinal villus length and egg quality was investigated.

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Ethics Committee Approval

13/04/2023 dated and numbered 2023/02 was given by Local Ethics Committee of Poultry Research Institute Directorate (TAE-HADYEK).

Author Contributions

Study planning AAK, MD; Performing the experiment AAK, MD; Making analyzes FT, BK; Evaluation AAK, MD, FT, BK; Writing the final report FT, BK; Article publishing efforts BK

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The Effect of Calving Season and Parity on Some Reproductive Performance in Anatolian Buffaloes Reared in Bartın Province

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ABSTRACT

The purpose of this research is to determine the effect of calving season and parity on the reproductive performances of CI (calving interval) and DP (dry period) in Anatolian buffaloes raised under farm conditions in Bartın province of Turkey between 2015 and 2020. For this purpose, 1251 records of 616 Anatolian buffaloes were evaluated to determine CI and DP from the reproductive performances of Anatolian buffaloes. In the analysis of the data obtained in the study, the effects of calving season and parity, which are environmental factors, on DP and CI, which are reproductive parameters, were calculated by the "Least Squares Method". Statistical significance of mean values was determined by analysis of variance, and statistical differences between means were evaluated using the Tukey test. Within the framework of the study, the values of CI and DP, the reproductive characteristics of Anatolian buffaloes, were determined as 423.84 ± 3.06 days and 149.55 ± 2.81 days, respectively. In the work, the effect of parity and calving season on CI and DP was found to be statistically significant. At the same time, high positive phenotypic correlations were found between CI and DP (r: 0.722, p<0.001). In this study, the highest reproductive performance in Anatolian buffaloes was obtained in the third and fourth parities, with those calving in the spring season. In the study, it was concluded that taking into consideration the CI and DP factors, which are found to be important and affecting productivity in the selection of breeding animals by breeders will contribute to farm efficiency.

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INTRODUCTION

Buffalo (Bubalus bubalis) is a dairy animal that is raised worldwide, especially in Asia, where 98 percent of the world's buffalo population can be found. India (54.5%), Pakistan (21.3%) and China (13.1%) are home to 88.9 percent of the world's buffalo population (FAO, 2022). The water buffaloes reared in Turkey are descended from Mediterranean buffaloes – a subgroup of river - type buffaloes. Referred to today as Anatolian buffaloes (Cicek et al., 2009), they are raised mostly in the northern, central, western, eastern and southeastern parts of Anatolia (Atasever and Erdem, 2008).

The population of Anatolian buffalo in Turkey had decreased from about 1.2 million head to 84,726 head between 1960s and 2010. Then, the "National Anatolian Buffalo Breeding Project in Farmer Conditions" was launched by the Ministry of Agriculture and Forestry (MoAF) in 2011, which resulted in the number of Anatolian buffalo in Turkey increasing to 161,749 head in 2020 (TURKSTAT, 2023).

Buffalo breeding in Turkey is generally focused on the production of meat and especially milk (quark, yogurt, cheese, ice cream). Foods of animal origin such as meat and milk play an important role in human nutrition (Oyan et al., 2024). Especially recently, there has been an increase in studies showing that whey proteins produced from milk can be used as an important food additive in the nutrition of infants, the elderly and sports (Aktan and Uçar, 2022). Milk yield and reproduction; it is influenced by factors such as age, genotype, season, management and nutrition (Kumar et al., 2017). CI is an indicator of the reproductive status of dairy animals. In cows and buffaloes, the recommended CI is 12-13 months, and if a dairy animal is to be cost-effective, it should have a shorter DP and a shorter CI. In this regard, CI and DP are important economic factors affecting milk productivity in buffaloes (Sanker et al., 2014).

The aim of this research is to define the effects of parity and season on the reproductive performance (CI, DP) of Anatolian buffaloes reared under farm conditions. It is believed that the information obtained from this study will help in the formulation of future breeding plans of Anatolian buffaloes.

MATERIAL AND METHOD

Study Area, Animals and Data Collection

The data were acquired from the "Manda Yıldızı" database (Tekerli, 2019) within the scope of the "Anatolian Buffalo National Breeding Project in Farmer Conditions" supported by the Directorate General of Agricultural Research and Policy (TAGEM). The research data included the pedigree records of 901 head of Anatolian buffalo reared in Bartın province (41° 38' N and 32° 20' E) between 2015 and 2020. In the study, 1251 records of reproductive performance of Anatolian buffaloes that calved between 2015 and 2020 were evaluated. In the region, buffalo husbandry is mostly practiced under pasture conditions. During the grazing season, buffaloes are generally not given supplemental feed, but are provided with small amounts of stock feed (dried alfalfa, straw, silage, etc.) in winter. Buffalo in the herd reproduce by natural mating. Buffalo in the herd reproduce by natural mating. Colostrum is given to newborns to provide passive immunity and contribute to the development of the gastrointestinal tract (Uysal et al., 2024). Milking is done twice a day, morning and evening, by hand for most farmers and by machine for the rest.

In the study, records with a CI of ≥300 and ≤700 days (Koçak et al., 2019) and a DP of ≥30 and ≤300 days (Poudel et al., 2017) were assessed. The findings obtained by Alkoyak and Öz (2020) in a study conducted in the same area were also used. The DP was calculated considering the date on

which the animals were dried off at the end of the ongoing lactation. In line with the prevailing geo-climatic conditions in Turkey, calving seasons are split into four groups; winter (1) (February, January, December), spring (2) (May, April, March), summer (3) (August, July, June), fall (4) (November, October, September). Parity was ranked numerically from 1 to 5.

Statistical Analysis

In this study, the effects of season and parity on reproductive parameters were calculated using the least squares method. Statistical significance of means was determined by analysis of variance, and differences were evaluated using the Tukey test. Due to lack of data in the subgroups, no two- or three-way interaction was assumed between the factors studied. In addition, the phenotypic correlation was determined using the Pearson's method. The general linear model within the Minitab version 18 package and the correlation procedure were used for the analyses (Minitab, 2017).

The following statistical model was used to calculate the effect of season and parity on CI and DP.

$Yi_jkl = \mu + S_i + P_j + H_k + e_{ijkl}$ where;

Y_{ijkl} is the production level of any buffalo (i. season, j. parity, k. observation level of the herd for the characteristic in question)

μ represents the mean (expected),

S_i represents the effect of calving season (i: 1, 2, 3, 4),

P_j represents the effect of parity (j:1, 2, 3, 4)

H_k represents the effect of herd (k: 1- 118 different herds)

e_{ijkl} represents the random error (NID, 0, σ2).

RESULTS

Means, standard errors and influential factors for the effect of season and parity on reproductive performance are calculated (Table 1). The number of herds examined (118 herds) is not given in Table 1 because it is too high. The results of the analysis of variance regarding the environmental factors examined in the study are given in Table 2. The effects of calving season and parity on DP and CI were significant ($p<0.01$, $p<0.001$). High positive phenotypic correlations were found between CI and DP (r: 0.722, $p<0.001$)

Table 1

Mean Least Squares (± Se) Of Ci and Dp of Anatolian Buffaloes According to Season And Parity

FACTORS	CI (Days)		DP (Days)	
	n	(Mean±SE)	n	(Mean±SE)
Overall average	1251	423.84±3.06	1251	149.55±2.81
Calving Season		**		**
Winter	195	448.32±5.56^{a}	195	164.85±5.10^{a}
Spring	282	413.88±4.99^{b}	282	142.24±4.58^{b}
Summer	436	417.04±4.35^{b}	436	150.03±3.99^{ab}
fall	338	416.12±4.46^{b}	338	141.09±4.09^{b}
Parity		*		**
1st	451	436.25±3.73^{a}	451	165.22±3.42^{a}
2nd	381	423.23±4.01^{b}	381	152.21±3.67^{b}
3rd	247	417.09±4.93^{b}	247	142.22±4.52^{b}
4th	172	418.78±5.92^{b}	172	138.55±5.43^{b}

*:p<0.01; **: p<0.001
a, b: Differences between the means in the same column with different superscripts are significant (p<0.01).

Table 2

Analysis of Variance of Factors Affecting Ci and Dp

Factors		CI		DP	
	D.F	M.S	F-Value	M.S	F-Value
Herd	113	11361	2.62**	7042	1.94**
Calving season	3	47247	10.91**	23285	6.40**
Parity	3	23639	5.46*	38848	10.68**
Error	1131	4330		3638	

M.S: Mean square; D.F: Degrees of freedom; *p<0.01; **p<0.001

Table 3

Phenotypic Correlation (Rp) for Ci and Dp of Anatolian Buffaloes

Traits	CI	DP
CI	-	
DP	0.722**	-

CI: Calving interval; DP: Dry period; **P<0.001

DISCUSSION

In the study, the mean CI found in Anatolian buffaloes (423.84±3.06 days) was similar to the findings of other researches (Alkoyak and Öz, 2020; Alkoyak et al., 2023; Malhado et al., 2013; Soysal et al., 2018), who reported CI's of 411 days in Murrah buffaloes and 411.3 - 426.3 days in Anatolian buffaloes. However, this value is higher than that of Marai et al., (2009) on Egyptian buffaloes (402.6 days). Conversely, there have been many studies (Komori et al., 1994; Tekerli et al., 2001; Thevamanoharan 2002; Hussain et al., 2006; Sanker et al., 2014; Charlini and Sinniah 2015; Nava-Trujillo et al., 2018; Koçak et al., 2019) reporting higher CI's than in this study, such as 453 days in Murrah buffaloes, 450.2–496 days in Nili-Ravi buffaloes and 441.9–450.3 days in Anatolian buffaloes.

In the present study, the effect of calving season on CI was determined to be significant (p<0.001). Consistent with this study, there are studies that reported the effect of calving season on CI to be important in Anatolian buffaloes (Tekerli et al., 2001; Koçak et al., 2019; Alkoyak and Öz 2020; Alkoyak et al., 2023) and Egyptian buffaloes (Marai et al., 2009). In contrast to the present study, the effect of season on CI was not found to be significant in the research of Anatolian buffaloes in İstanbul (Soysal et al., 2018). While in the present study the longest CI for Anatolian buffaloes was observed in animals that calved in winter, the shortest CI was observed in animals that calved in summer, and other studies have obtained similar results (Bashir et al., 2015; Koçak et al., 2019), which supports the results of this study. The fact that the shortest CI was observed in animals that calved in summer could be attributed to the fact that the gestation period of the animal after birth could continue into the fall or winter seasons. In winter and fall, the reduce in day length and the decrease in temperature may lead to an increase in sexual activity in buffaloes. In this work, the effect of parity on CI was significant (p<0.01). Similarly, Soysal et al. (2018), Alkoyak and Öz (2020), and Alkoyak et al. (2023) found that

the effect of parity on CI was significant in Anatolian buffaloes, and there are many other studies (Charlini and Sinniah, 2015; Fakruzzaman et al., 2020; Hussain et al, 2006; Marai et al., 2009; Nava-Trujillo et al., 2018; Sanker et al., 2014) that reported the effect of parity on CI to be significant in buffaloes. In the research, it was observed that CI steadily decreased with increasing parity, and this result was similar to the findings of other studies (Charlini and Sinniah, 2015; Alkoyak and Öz, 2020), which may be attributable to the lower reproduction performance of buffaloes at early parity, and the increase in their performance with increased age. Different from this study, Tekerli et al., (2001) conducted parity to have no significant effect on CI.

In the present study, the mean DP of Anatolian buffaloes was 149.55±2.81 days, which was similar to the DP found in other studies (Marai et al., 2009; Sanker et al., 2014), which were 144.3 days for Nili-Ravi buffaloes and 148.7 days for Egyptian buffaloes. However, there have been studies (Alkoyak et al., 2023; Charlini and Sinniah, 2015; Hussain et al., 2006) that reported longer DP's than those in the present study, such as 179-194.4 days for Nili-Ravi buffaloes and 191.7 days for Anatolian buffaloes. There are also studies (Poudel et al., 2017; Verma and Yadav 1989) reporting shorter DP's than those in the present study, e.g. 110.9 days for crossbred Murrah buffaloes and 120 days for Nili-Ravi buffaloes. However, if a dairy animal is to be cost effective, it must have a shorter DP (Poudel et al., 2017). DP is the necessary resting period between calving and animals normally have low yield during this period. When this period is longer than normal, milk production decreases, which affects the economics of the farm. In this work, the effect of calving season on DP was reported to be significant ($p<0.001$). Similarly, Alkoyak et al. (2023) and Hussain et al. (2006) found that DP was significantly affected by calving season. On the other hand, Thevamanoharan (2002) found that calving season had no significant effect on DP in Nili-Ravi buffaloes, unlike the present study. Furthermore, the effect of parity on DP was found to be significant ($p<0.001$). Similarly, there have been studies (Hussain et al., 2006; Sanker et al., 2014; Alkoyak et al., 2023) that reported the effect of parity on DP to be significant, thus supporting the findings of this study. Contrary to the findings of this study, there have been studies (Hussain et al., 2006; Poudel et al., 2017; Fakruzzaman et al., 2020) reporting parity to have insignificant effect on DP. In this work, the highest DP was measured in the first parity, with a steady decrease observed in later parities (Table 1). Similarly, there have been studies (Fakruzzaman et al., 2020) reporting that the longest drying period was observed in buffaloes in the first parity, and a significant decrease in the following parities.

This study also found positive, high and significant correlations ($r= 0.722$, $p<0.001$) between the reproductive traits of CI and DP (Table 3), and similarly, there are other studies reporting high and significant phenotypic correlations between these traits (Marai et al., 2009; Galsar et al., 2016; Jakhar et al., 2016). On the other hand, Aziz et al. (2001) reported only moderate phenotypic correlation between CI and DP. In the present study, the investigated environmental factors were found to influence the reproductive traits of CI and DP. Since high and positive phenotypic correlations were observed, it can be concluded that reproductive traits could be improved by better control of the identified environmental factors.

CONCLUSION

It can be concluded that if the Anatolian buffalo breeders take the necessary measures in terms of care, nutrition and herd management, their reproductive performance will increase. The significant correlations between CI and DP indicate that buffalo breeders can improve reproductive traits by ensuring better management conditions in their farms. In this study, the highest reproductive performance of buffaloes was obtained in the third and fourth parity with those calving in spring. It was concluded that taking into account the factors considered important and affecting the yield in the selection of breeding animals and using these factors in the genetic improvement of animals, could contribute significantly to farm productivity.

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Ethics Committee Approval

In our study titled "The effect of calving season and parity on some reproductive performance in Anatolian buffaloes raised in Bartin province", no action was taken on the animals and only the data recorded in the breeding project were evaluated. The authors declare that an ethics committee decision is not required.

Authors Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Sezer Öz, Kürşat Alkoyak and İrfan Güngör. The first draft of the manuscript was written by Kürşat Alkoyak and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Sustainable Development Goals (SDG): 12 Responsible Consumption and Production, 15 Life on Land

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Laminitis in Cattle: A Bibliometric Analysis

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ABSTRACT

Laminitis is a condition characterized by lameness, which poses a significant economic burden on the dairy industry. The condition presents a challenge in combat due to its multifactorial etiology. This is why it remains a widespread issue globally, including the developed countries. This study aims to reveal publication trends and interactions between studies outcomes based on the data obtained from the Web of Science. To access the appropriate articles, a search was conducted using the keyword "laminitis" as the inclusion criteria. The search criteria included selecting "English" as the language, "Article" as the document type, and "All Fields" as the search method. The study revealed that a total of 967 articles were published during the analyzed period. The average annual growth rate of the studies was found to be 6.35%. The highest number of articles were published in 2019 with 64 articles (6.61%). With the keyword analysis, "lameness", "cattle," and "dairy cattle" were identified as the most relevant keywords. The analysis of the keywords indicated that laminitis is an important concern not only for animal welfare but also for behavioral sciences as well as economic loss. In terms of article count, the United Kingdom exhibited the highest performance. The journal with the highest number of articles in this field was Preventive Veterinary Medicine 8.8% (85 articles). Whay H.R. from the United Kingdom was identified as the most influential author. A general upward trend in the number of publications primarily driven by developed countries was observed during the period analyzed in the study.

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INTRODUCTION

Laminitis, also referred to as diffuse aseptic pododermatitis, is inflammation occurring within the dermal layers of the hoof (Belge et al., 2005; Momcilovic et al., 2000) Numerous factors including age, parturition, lactation, and milk production contribute to the development of laminitis (Orhun et al., 2023). Among them, excessive intake of fast-digesting carbohydrates stands out as a primary factor (Şenyüz and Karslı 2021a, 2021b). As a metabolic consequence of ruminal acidosis, degeneration of the dermo-epidermal junction occurs in the laminar region of the hoof. Many studies have examined the relationship between acidosis and laminitis in cattle (Danscher et al., 2009; Ding et al., 2020). Laminitis in cattle manifests in four distinct forms: acute, subacute, chronic, and subclinical (Altuğ et al., 2019; Bergsten, 1994). Subclinical laminitis is more difficult to detect compared to other forms of laminitis because movement changes are not observed despite physical changes in the hoof (Smilie et al., 1996).

Nowadays, laminitis causes significant economic challenges primarily due to its impact on milk production. Mastitis, reproductive issues, and laminitis stand out as significant contributors to milk production losses. Laminitis can result in weight loss, reduced milk production, disturbances in the reproductive cycle, and ultimately, culling. In addition to these, treatment costs also increase economic loss (Burger, 2017). In a study conducted in the UK, it was reported that laminitis accounted for 27% of the cost attributed to production diseases during the 1992-1993 breeding season (Kossaibati and Esslemont, 1997). Apart from economic losses, laminitis inflicts pain on the animals, which leads to a significant decline in their welfare. Controlling laminitis is challenging because it doesn't exhibit symptoms during the subclinical stage (Bell and Weary, 2000).

Bibliometrics is a statistical method utilized to review a particular field of literature, assess its progression, and identify future trends (Miao et al., 2022). Different research methods need to be used together to provide a holistic understanding of any given topic. Therefore, this study aims to define and map the concept of laminitis using the bibliometric analysis technique.

MATERIALS AND METHODS

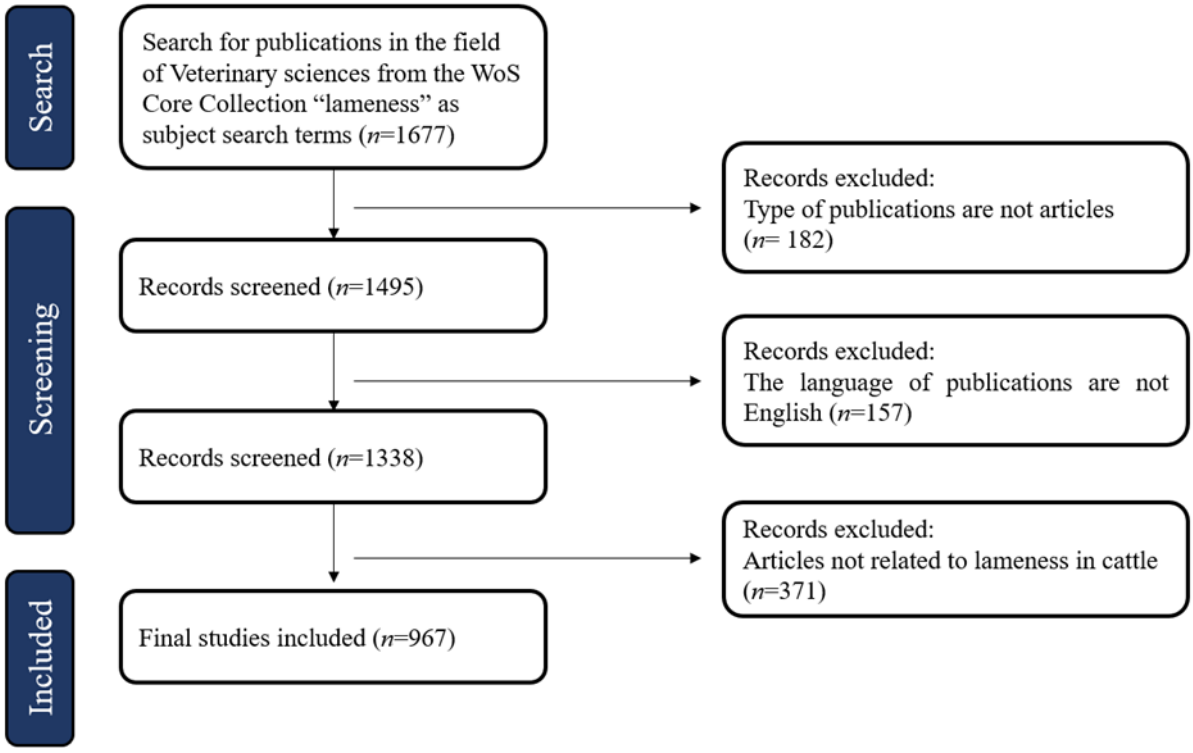
Database and Search Technique

Before designing a bibliometric study, the first step is to identify a proper database and an effective search strategy. In the current study, the Web of Science (WoS) database was employed. WoS is a widely used database in the academic community, known for its reliability, validity, and accuracy (Pranckutė, 2021; Sevinc, 2004).

The search criteria included selecting “English” as the language, “Article” as the document type, and “All Fields” as the search method. The studies retrieved from this search were thoroughly examined to enhance the reliability of the data. The data for this study was retrieved from the WoS core collection on April 10, 2024, using the inclusive term “laminitis” as the search query. The search yielded 1677 studies in the field of veterinary sciences. After applying article and language restrictions, a total of 1338 studies were achieved. After individually reviewing the studies, those that did not align with the subject matter and did not involve the cattle species were identified and excluded from the database. Finally, 967 studies were included in the analysis. (Figure 1).

Figure 1

Bibliometric Dataset Acquisition Process



Bibliometric Research Methodology

In general, a bibliometric analysis comprises performance analysis and science mapping. Performance analysis reveals the contributions of research elements to the field, whereas science mapping reflects the relationships between these research elements. The effective integration of these two methods reveals both the structural and intellectual relationships within the research area under investigation (Baker et al., 2021; Tunger and Eulerich, 2018). Bibliometric studies typically assess parameters such as publication count, citation count, collaboration index, and h-index. Other evaluation criteria may also be taken into consideration (Aria and Cuccurullo, 2017).

RESULTS

Between 1980 and 2024, a total of 967 eligible research articles were published across 106 journals, involving 2872 authors. According to the author/article analysis, the average number of authors per article, the average number of articles per author, and the average number of co-authors per article were 2.97, 0.33, and 4.26, respectively. The numbers of single-authored and multi-authored articles were found to be 72 and 895, respectively. The collaboration index was determined to be 3.13 (Table 1).

Table 1

Overview of the Laminitis Studies

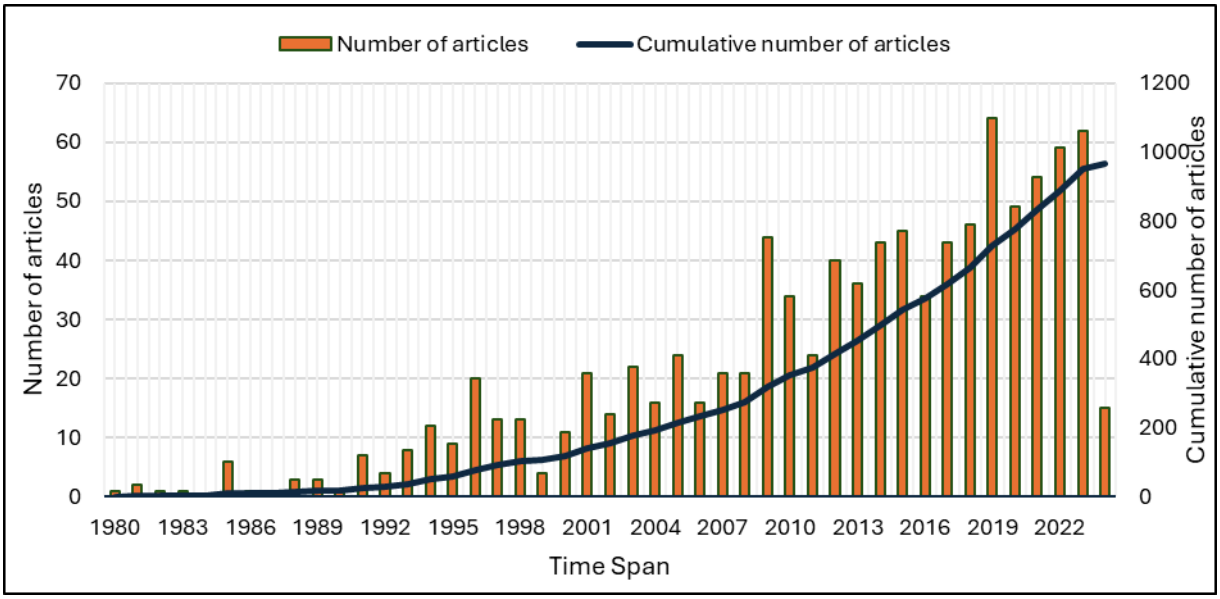
Description	Results
Main Information About Data	
Timespan	1980:2024
Sources (Journals)	106
Documents	967
Annual growth rate %	6.35
Document average age	11.8
Average citations per document	23.41
References	16273
Document Contents	
Keywords Plus (ID)	1585
Author's Keywords (DE)	1841
Authors	
Authors	2872
Author appearances	4119
Authors of single-authored documents	62
Authors of multi-authored documents	2810
Authors Collaboration	
Single-authored documents	72
Multi-authored documents	895
Authors per document	2.97
Documents per author	0.33
Co-Authors per document	4.26
Collaboration index	3.13
Author footprint index	0.31
International co-authorships %	20.17
Document Types	
Article	917
Article; early access	2
Article; proceedings paper	48

The annual statistics of the publications about laminitis are given in Figure 2. An average

annual increase of 6.35% was observed in the number of research articles during the research period. The highest number of publications about laminitis was observed in 2019 with 64 articles (6.61%). Unlike other diseases, laminitis necessitates specialized treatment and preventive measures. It leads to significant economic losses for producers because it is influenced by all practices related to nutrition, housing, and yield (Tsousis et al., 2022). The observed increase in the publication count on laminitis can be explained by these factors and growing interest in the subject over time.

Figure 2

Annual Number of Publications on Laminitis (1980 – 2024)



Top Publishing Journals

Table 2 displays the top 10 most influential journals identified through the performance analysis. During the research period, a total of 967 research articles were published across 106 journals. The top 3 most influential journals were Preventive Veterinary Medicine 8.8% (85 articles), Animals 8.4% (81 articles), and Veterinary Journal 6.5% (63 articles) respectively. Upon detailed examination, it was discovered that these journals are open-access and consistently publish studies of high clinical quality in the field of veterinary sciences.

Table 2

The Top 10 Most Influential Journals on Laminitis

Rank	Journal	Frequency (%)
1.	Preventive Veterinary Medicine	85 (8.8)
2.	Animals	81 (8.4)
3.	Veterinary Journal	63 (6.5)
4.	Cattle Practice	59 (6.1)
5.	Veterinary Record	58 (6.0)
6.	Veterinary Clinics of North America-Food Animal Practice	41 (4.2)
7.	Animal	37 (3.8)
8.	Applied Animal Behaviour Science	34 (3.5)

9.	Animal Welfare	31 (3.2)
10.	Frontiers in Veterinary Science	29 (3.0)

Analysis of Authors

The survey data included 2872 authors who published 967 articles between 1980 and 2024. Among these authors, 62 published single-authored and 2810 multi-authored articles. As seen in Table 3, the top 3 most influential authors according to the h-index were Whay H.R. (17), Main D.C.J. (13), and Thomsen P.T. (13), respectively. Whay H.R., the author with the highest publication and citation counts on this subject, placed particular emphasis on animal welfare and behavior in dairy cattle. Since laminitis is an important disease that impairs animal welfare in dairy cattle, numerous studies have been conducted to better understand and address this important disease (Whay and Shearer, 2017). "Assessment of the welfare of dairy cattle using animal-based measurements: direct observations and investigation of farm records" published in 2003 and co-authored by Whay H.R., Main D.C.J., and Green L.E., has received 721 citations (Whay et al., 2003). This indicates high collaboration among the top 10 most influential authors.

Table 3

Top 10 Most Influential Authors on Laminitis

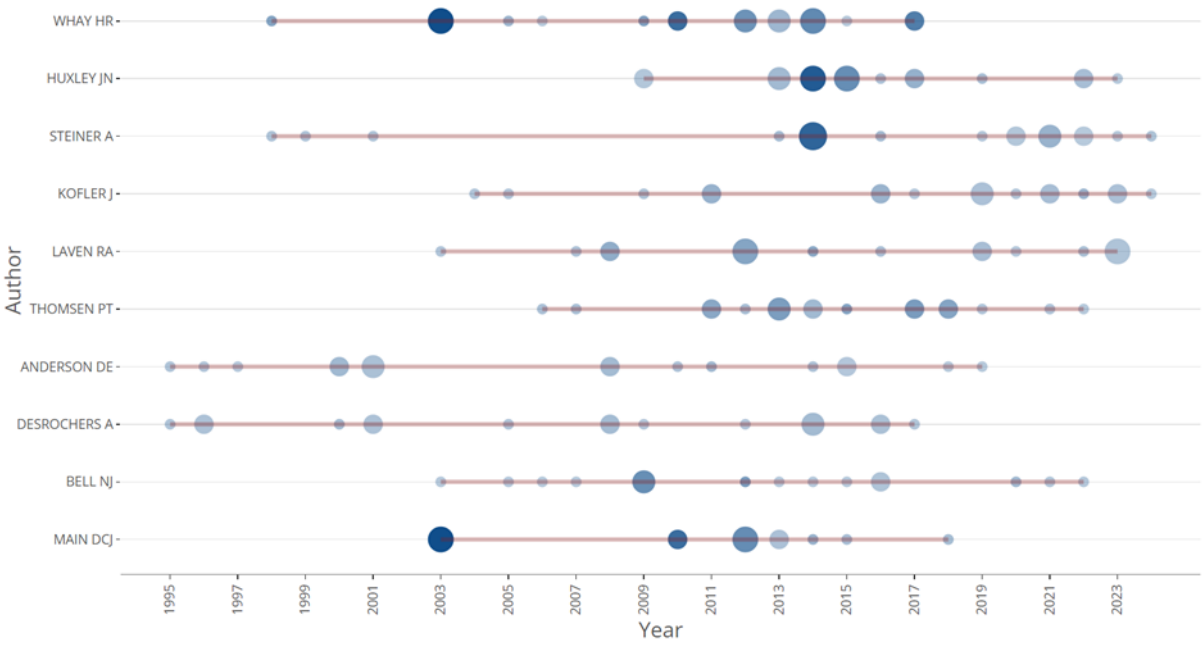
Authors	h-index	g-index	m-index	TC	TP	FPY
Whay HR	17	23	0.63	1485	23	1998
Main DCJ	13	15	0.59	963	15	2003
Thomsen PT	13	18	0.68	480	18	2006
Green LE	12	14	0.43	977	14	1997
Anderson DE	11	16	0.37	278	17	1995
Murray RD	11	13	0.34	1064	13	1993
Bergsten C	10	12	0.31	754	12	1993
Desrochers A	10	16	0.33	278	17	1995
Huxley JN	10	20	0.63	425	20	2009
Leach KA	10	10	0.36	624	10	1997

TP: Total Publications, TC: Total Citations, FPY: First Publication Years

Figure 3 illustrates the productivity of the top 10 most influential authors over time. Notably, the most influential author Whay H.R. published 4 articles in 2003, with an average of 21.86 citations per article. Among the most recent studies, Laven R.A. stands out with 4 articles published in 2023.

Figure 3

The Productivity of the Most Influential Authors on Laminitis Over Time



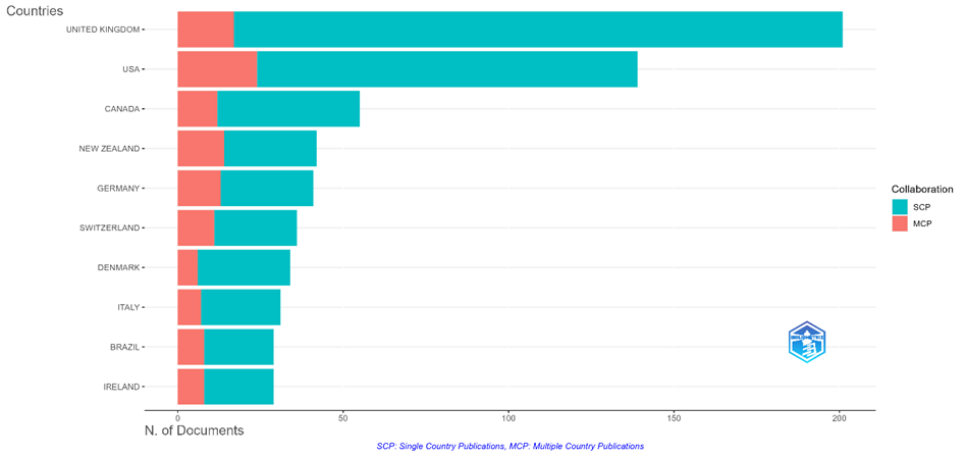
Note: The size of the nodes corresponds to a higher publication count, while the darkness of the color indicates a higher citation count.

Country Performances

Globally, 62 countries have contributed to the research on laminitis since 1980. The distribution of publication count of the corresponding authors' countries is shown in Figure 4. The top 10 countries include countries from different continents, including the United Kingdom, the United States of America (USA), New Zealand, and Brazil. The United Kingdom (201) was identified as the country with the highest number of publications on laminitis. The USA (24) had the highest multiple-county article count. New Zealand (33.3%) was identified as the country with the highest multiple-county article ratio. No country has exceeded an international collaboration rate of 50%. However, some countries showed stronger domestic collaborations. For example, the two most influential authors, Whay H.R. and Main D.C.J., were from the United Kingdom and have collaborated on some articles (Whay et al., 2003).

Figure 4

Publication Counts of the Corresponding Authors' Countries

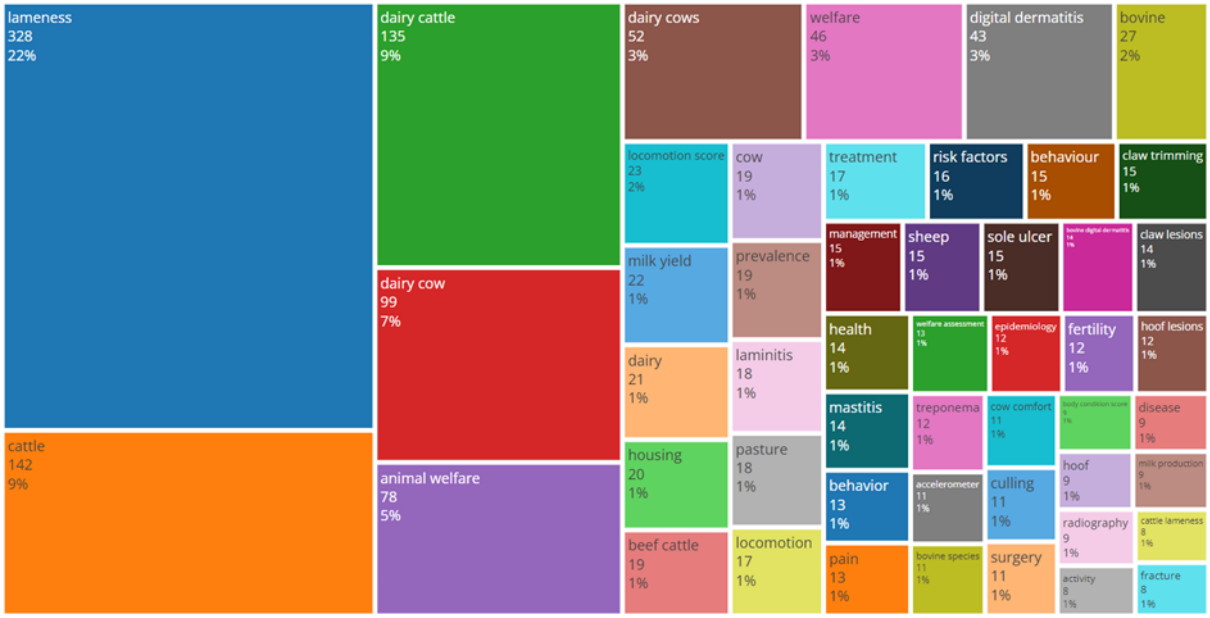


Co-Word Analysis

The tree map of high-frequency keywords occurring in the laminitis studies is shown in Figure 5. The most frequent 5 keywords were lameness 328 (22%), cattle 142 (9%), dairy cattle 135 (9%), dairy cow 99 (7%), and animal welfare 78 (5%), respectively. It was observed that "dairy cattle" (or dairy cow) is a frequently used keyword in laminitis studies involving cows. This finding can be attributed to the lactation and longer lifespan characteristic of dairy cattle (Thomsen et al., 2023). Since laminitis is associated with many factors, various keywords from diverse disciplines such as "animal welfare," "pain," "mastitis," "digital dermatitis," "surgery," "pasture," "fertility," and "management" frequently occur.

Figure 5

The Tree Map of the High-Frequency Keywords Occurring in Laminitis Studies



DISCUSSION

Laminitis in cattle results in substantial economic losses, particularly within the dairy farming sector. This is why studies on factors causing laminitis are still conducted globally. The protection measures developed can be implemented to varying extents depending on the modern production opportunities of the cattle farms in country or region. The protective measures developed can be

implemented to different extents depending on the modern production opportunities of cattle farms, as well as the country or region (Garvey, 2022). Since laminitis remains a significant challenge for dairy farmers, this study presented a bibliometric analysis to assess the intellectual development of studies in the field. The annual growth rate of studies on laminitis was determined to be 6.35%. A notable surge in the number of studies on laminitis was observed in 2009 and 2019. Furthermore, despite occasional decreases in certain years, there is an overall linearly increasing trend in the number of publications. Thomsen et al. reviewed 53 laminitis studies from 6 continents, with a predominant focus on Europe and North America, over a 30-year period. In that study involving 414950 cows from 3945 herds, the prevalence of laminitis ranged between 5.1% and 45%, with an average prevalence of 22.8% (Thomsen et al., 2023). According to the European Food Safety Authority (EFSA) Panel on Animal Health and Animal Welfare, a prevalence of detectable locomotor difficulties in dairy cattle exceeding 10% indicates that the prevention program is inadequate (EFSA, 2009). The significant challenge of combating laminitis has drawn the attention of researchers in the field. Through their collaboration, researchers from the United Kingdom, including Whay H.R., Main D.C.J., and Green L.E., have positioned their country as the most influential in this field. Brazil, a country with a high cattle population, has also ranked among the top 10 most influential countries in this field. Among the journals publishing laminitis studies, Preventive Veterinary Medicine, Animals, and Veterinary Journal were identified as the journals in the laminitis field with the highest impact factor. Studies in the field of laminitis are therefore of global interest to journals. Moreover, this global interest also encourages researchers to conduct new studies to explore potential treatment and prevention methods for this lesion. The fact that the most frequently occurring keywords in the field were from different veterinary sciences indicates that laminitis is a multifactorial disease. Besides, this finding also highlights the importance of multidisciplinary collaboration.

CONCLUSION

This study analyzed the literature on the laminitis using the data obtained from the WoS database. Accordingly, the most influential authors and journals, as well as the geographical distribution of the studies were identified and presented through tables and graphics. The findings indicated a rising trend in the number of research articles between 1980 and 2024. The analysis of the keywords indicated that laminitis is an important concern not only for animal welfare but also for behavioral sciences as well as economic loss. The results of this study offer a template for identifying hot research topics and future trends. Besides, this study allows for assessing the impact of the laminitis studies. This topic is likely to receive increased attention, particularly if research institutions are supported with proper policies and resources aimed at fostering studies on laminitis. In addition, co-operation between countries and studies on the effectiveness of preventive measures to combat laminitis should be carried out.

Strength and Limitations

This study serves as a guiding resource for researchers with an interest in laminitis. However, there are certain limitations to consider. Firstly, the review of laminitis studies relied solely on data from a single database. Nevertheless, the use of WoS provided a high-quality dataset, as it includes a larger number of articles in this field compared to Scopus or PubMed. Only research articles in English were included in the dataset. Additionally, it's important to note that since WoS counts an article once for each author, certain countries' statistics might be inflated. However, the articles obtained by the search were meticulously reviewed to ensure their direct relevance to the subject matter, and studies deemed unsuitable were excluded from the analysis. Despite these limitations, the findings offer sufficient evidence to reveal the research trends of authors and countries in the field of laminitis and to track the development of the field over time.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Hakan SERİN and Muslu Kazım KÖREZ. The first draft of the manuscript was written by Hakan SERİN and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

There is no conflict of interest between the authors.

Sustainable Development Goals (SDG): 3 Good Health and Well-Being

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