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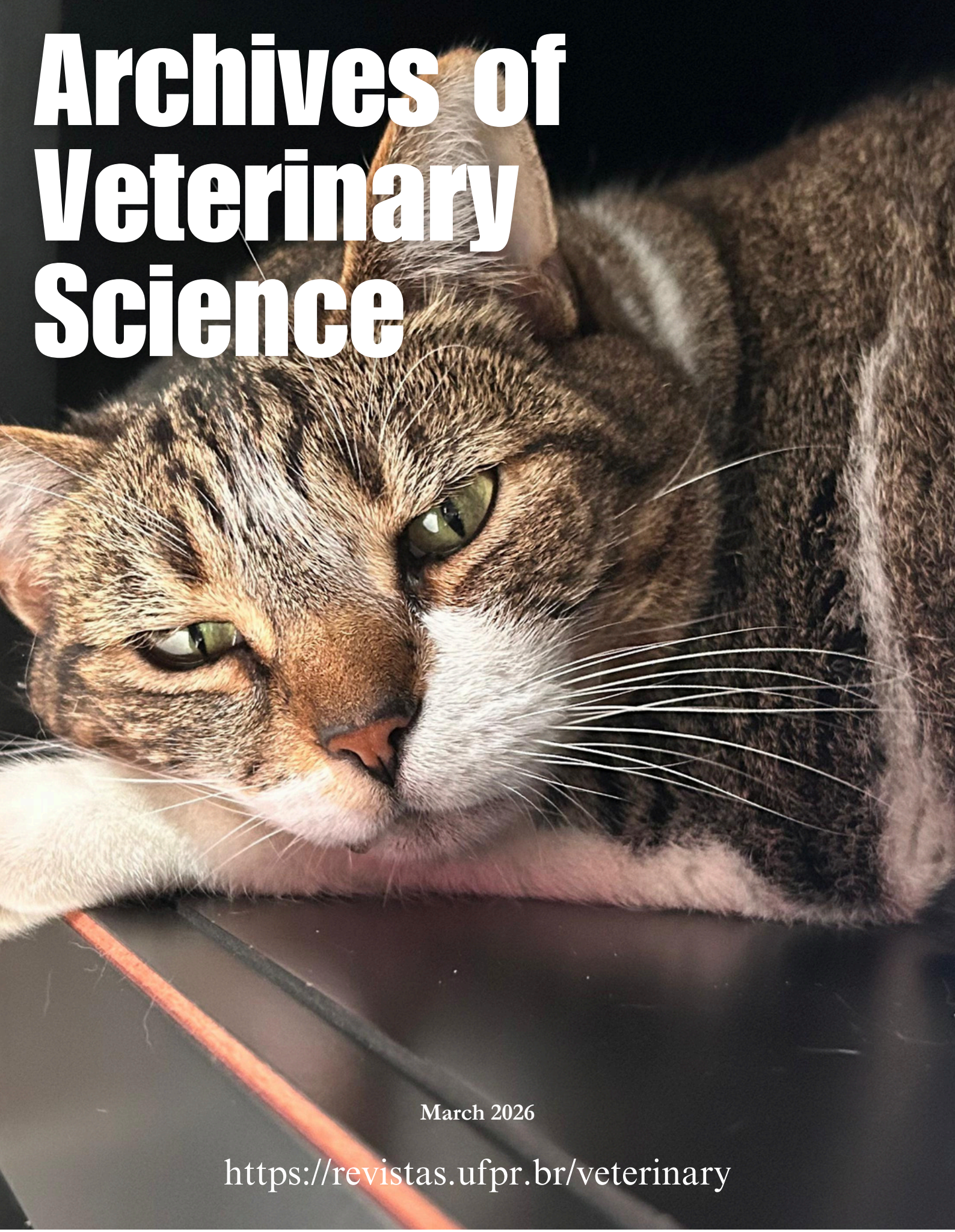

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Eimeria leuckarti in equids from properties in the Zona da Mata of Minas Gerais

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Abstract: Brazil has a significant population of equids, which makes the equine industry an important contribution on the national Gross Domestic Product (GDP). Parasitic diseases are prevalent, particularly those affecting the gastrointestinal tract, which can cause colic, diarrhea, and, in severe cases, result in fatality. Among these diseases, the protozoan *Eimeria leuckarti*, a parasite of the small intestine in horses, mules, and zebras, predominantly affects foals. When *E. leuckarti* causes clinical disease, symptoms such as acute and chronic diarrhea, abdominal pain, colic, and urticaria may occur, although it often presents asymptomatically. The present study analyzed 247 foals from eight stud farms located in the Zona da Mata of Minas Gerais (ZMM) region, encompassing the cities of Coimbra, Guaraciaba, Ponte Nova, Ervália, São Geraldo, Ubá, Teixeiras, and Viçosa. Data were collected through questionnaires administered to owners regarding the animals' production history and characteristics, and coproparasitological examinations were conducted using the Sheather technique at the Laboratory of Parasitology and Parasitic Diseases of the Veterinary Department of the Federal University of Viçosa. The collected data were organized into Microsoft Excel spreadsheets and subjected to statistical analysis using IBM SPSS Statistics software, version 25, applying Measures of Association tests and Yates-corrected Chi-Square tests, with a significance level set at $p < 0.05$. The prevalence of *E. leuckarti* in the ZMM region was 7.3%, and silage feeding was identified as a risk factor for eimeriosis in foals ($p < 0.01$). Other factors, such as feed type, housing conditions, and fecal disposal, did not show statistically significant differences.
Keywords: Coccidiosis. Eimeriosis. Parasitic diseases.

1. Introduction

Brazil has an extensive equine herd, estimated at around 5.8 million animals, with Minas Gerais having the largest population, accounting for approximately 804,000 equids (IBGE, 2025). This sector creates around 3 million direct and indirect jobs and generates annual revenue of 30 billion reais (Lima, 2016). Historically, equids have played crucial roles in social, agricultural, sporting, leisure, and therapeutic activities (Nascimento and Nardi, 2021).

Currently, the breeding of horses for leisure, sports, and rural tourism has outpaced their traditional work roles (Tomljenovic, Boranic-Zivoder, and Corak, 2018). Horses bred for sports and leisure require more intensive care and a greater investment, mainly because of their participation in events and competitions (Lima, 2016). In this context, health management practices, sanitary care, such as deworming and vaccination, is essential to maintaining the herd's health (Guelpa, 2023).

Parasitism poses a significant challenge to equine breeding, affecting both horses kept in the field and those housed in stables, causing problems such as colic, diarrhea, and, in severe cases, death (Afonso, 2016). Although cyathostomins are the leading cause of gastrointestinal parasitosis in equines, coccidian protozoa, such as Eimeria spp., can also affect the digestive tract of these animals, especially foals (Bauer, 1988). *Eimeria leuckarti* is a cosmopolitan protozoan that parasitizes the small intestine of horses, mules, and zebras, but its detection is rare due to the high frequency of asymptomatic infections (Studzińska, Tomczuk, and Sadzikowski, 2008; Dubey and Bauer, 2018).

In the Zona da Mata of Minas Gerais (ZMM), the equine industry plays a significant role in the local economy and culture, contributing to the region's agricultural and sports activities. The health of equids directly affects their productivity and well-being. *Eimeria leuckarti* is an important etiological agent of coccidiosis in foals, potentially causing diarrhea and resulting in production losses. Understanding the prevalence of this parasite and the associated risk factors is essential for developing effective control and prevention strategies, thereby improving sanitary management practices, reducing economic losses, and enhancing animal welfare. This study aimed to determine the prevalence of *E. leuckarti* in equids in the ZMM and to identify the risk factors associated with infection. Fecal samples were collected from foals on various properties in the region and subjected to coproparasitological analysis to diagnose coccidiosis. Additionally, a questionnaire was administered to gather information on management practices and environmental conditions, and the data obtained were statistically analyzed to determine the prevalence and associated risk factors.

2. Materials and Methods

2.1. Ethics

This study was conducted with the approval of the Animal Use Ethics Committee (CEUA) of the Federal University of Viçosa (UFV), under protocol number 50554278388. The procedures were performed following the Code of Conduct for the Use of Animals in Teaching, Research, and Extension of the Department of Veterinary Medicine (DVT). A questionnaire was also administered to the owners or those responsible for the animals' maintenance to collect data on the association between risk factors and infection with the pathogen. The Human Research Ethics Committee (CEP) of the Federal University of Viçosa approved the questionnaire under protocol number 59014622.7.0000.5153.

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2.2. Animals and Sample Collection

Sample collection took place at stud farms located in the ZMM, in the micro-regions of Ubá, Viçosa, and Ponte Nova, specifically in the municipalities of Coimbra (Farm 1) (20° 50' 58" S, 42° 47' 28" W), Guaraciaba (Farm 2) (20° 33' 28" S, 43° 0' 22" W), Ponte Nova (Farm 3) (20° 24′ 57″ S, 42° 54′ 32″ W), Ervália (Farm 4) (20° 50' 25" S, 42° 39' 8" W), São Geraldo (Farm 5) (6° 40' 60" S, 44° 33' 0" W), Ubá (Farm 6) (21° 07′ 12″ S, 42° 56′ 34″ W), Teixeiras (Farm 7) (20° 39' 8" S, 42° 51' 31" W), and Viçosa (Farm 8) (20° 44′ 41″S, 42° 50′ 31″ W). Seven of the properties specialized in breeding Mangalarga Marchador horses, and one in donkeys and mules. The coproparasitological analyses were conducted at the Laboratory of Parasitology and Parasitic Diseases of the Department of Veterinary, Federal University of Viçosa (DVT/UFV).

Fecal samples were collected directly from the rectal ampulla of 247 animals (5 mules, 9 donkeys, and 233 equids) up to 12 months of age, from eight equid-breeding farms. The distribution of animals per property was based on their availability up to the first year of age (Table 1). To determine the sample size, the average prevalence reported by Dubey and Bauer (2018) across 77 global studies was calculated, yielding an estimate of 13.04%. The OpenEpi (https://www.openepi.com/Menu/OE_Menu.htm) was used to calculate the required sample size based on the average frequency, with a 99% confidence interval, resulting in an approximate sample size of 241 animals for the study.

Fecal samples were collected by restraining of the foals using halters or stocks (when available on the property). Using latex or silicone EVA gloves lubricated with mucilage, fecal samples were collected directly from the rectal ampulla, labeled, and stored under refrigeration. The owners or those responsible for the establishments answered a questionnaire, reporting the animal's history, housing conditions, feeding, cleaning, and health care.

Stud Farms	City	Nº of Samples
1	Coimbra	4
2	Guaraciaba	14
3	Ponte Nova	49
4	Ervália	10
5	São Geraldo	54
6	Ubá	92
7	Teixeiras	12
8	Viçosa	12

Table 1 – Distribution of the number of animal samples collected at each visited stud farm.

2.3. Coproparasitological Diagnostic

The coproparasitological diagnoses was performed at the Laboratory of Parasitology and Parasitic Diseases of the Department of Veterinary Medicine at the Federal University of Viçosa, using Sheather's flotation technique (Sheather, 1923) for oocyst visualization. This technique consists of centrifugal flotation procedure. In this method, the diluent solution used is a saturated sucrose solution with a density of 1.27 mg/mL (Sheather, 1923). Five grams of feces were weighed using a mini digital LCD scale, diluted in distilled water, and filtered through a 4-mesh gauze sieve. Subsequently, 10 mL of the mixture was transferred to a Falcon tube, and 10 mL of Sheather's solution, prepared in the laboratory (500g of sugar in 320ml of distilled water with 6g of phenol for preservation), was added. Centrifugation was performed at 2000 g for 5 minutes. After centrifugation, Sheather's solution was added dropwise until a meniscus was formed, where a glass slide was placed to capture the floating oocysts. The oocysts were identified using an optical light microscope (CX3) at 40x magnification.

2.4. Calculation of *E. leuckarti* prevalence

To calculate the prevalence of eimeriosis in the equids of the present study, the positive cases from each farm were divided by the sample size of the same and multiplied by 100, obtaining the percentage for each property, according to the following formula:

$$\text{Prevalence} = \frac{\text{number of positive cases on the farm}}{\text{number of animals on the farm}} \; x \; 100$$

To calculate the total prevalence data,the total number of positive cases was divided by the total number of animals collected and multiplied by 100, according to the formula:

$$\text{Total prevalence} = \frac{\text{total number of positive cases}}{\text{total number of animals in the experiment}} \; x \; 100$$

2.5. Statistics

The data were stored in Microsoft Excel 2016 and analyzed using IBM SPSS Statistics version 25. Descriptive analyses were performed, with relative and absolute frequencies presented as percentages. Measures of Association and Yates-corrected Chi-Square tests were used to assess the association between the positivity rate for *E. leuckarti* infections and potential risk factors for

infection, such as age, diarrhea, diet, fecal disposal, and housing. The strength (degree) of the association between infection prevalence and associated factors was assessed using the Risk Ratio (RR) calculated through on the OpenEpi website, in order to better understand the relationship between infection exposure and the risk factors to which the animals were exposed. A significance level of 0.05 was adopted.

3. Results

Coprological examinations identified, positive samples for *E. leuckarti* were identified (Figure 1). Based on the number of positive tests, the overall prevalence of infection with this parasite on properties within the ZMM was determined to be 7.3%. Additionally, prevalence was calculated for each property: Stud Farm 1 (Coimbra) showed a prevalence of 7.1%, Stud Farm 3 (Ponte Nova) had 14.3%, and Stud Farm 6 (Ubá) had 10.9%. No confirmed cases of *E. leuckarti* were found on the other stud farms (Table 2).

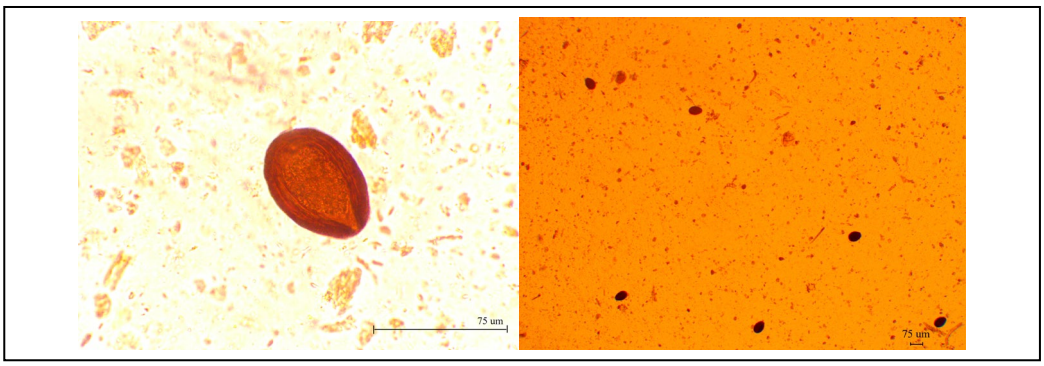

Figure 1 – *Eimeria leuckarti* diagnosed using the Sheather's method. (A) Image magnified 100x, showing the thick wall of the coccidian; (B) Image magnified 10x, displaying six oocysts of *E. leuckarti*.

An analysis was also conducted to assess the association between property risk factors and the occurrence of *E. leuckarti* infection in foals, using data from questionnaires administered to owners of the visited stud farms. Considering the fecal-oral nature of transmission, dietary characteristics, management practices, and the presence of diarrheal cases were analyzed for potential correlations (Table 3).

Stud Farms	N° of animals	Positive (%)
1	4	0 (0%)
2	14	1 (7,1%)
3	49	7 (14,3%)
4	10	0 (0%)
5	54	0 (0%)
6	92	10 (10,9%)
7	12	0 (0%)
8	12	0 (0%)
Total	247	18 (7,3%)

Table 2 – Prevalence of *E. leuckarti* in stud farms in the Zona da Mata Of Minas Gerais region.

Risk Factor	RR (IC 95%)	P-Value	EFP	EFE
Age	2,333 (0,9 - 6,0)	0,0585	38,1%	57,14%
Feeding				
Silage	12,78 (1,7 - 94,0)	0,0010**	87,05%	92,18%
Sugarcane	2,09 (0,8 - 5,2)	1,884	31,88%	52,16%
Ration	5,073 (3,1 – 8,7)	0,248	75,09%	80,29%
Grass	0,3049 (0,2 – 0,3)	0,241	52,13%	69,51%
Diarrheia	1,859 (0,7 - 4,5)	1,149	17,96%	46,2%
Destination of feces				
Pasture	0,02926 (0,009 - 0,071)	0,574	34,95%	97,07%
Hay	0,1438 (0,094 - 0,212)	0,477	38,53%	85,62%
Weeder	2,767 (2,1-3,5)	0,751	26,11%	63,86%
Composting / Other	5,88 (4,5 - 7,5)	0,751	49,39%	82,99%
Animal acesses				
Stall	41,62 (29,62 - 59,76)	0,061	82,98%	97,6%
Piquet	0,5819 (0,33 - 0,95)	0,983	4,181%	41,81%

Table 3 – Factors associated with eimeriosis in foals from properties in the Zona da Mata of Minas Gerais region. (RR - Risk Ratio; CI - Confidence Interval; EFP - Etiological Fraction in the Population; EFE - Etiological Fraction in the Exposed; P-value** p ≤0.01)

4. Discussion

The prevalence of *Eimeria leuckarti* on farms in the Zona da Mata of Minas Gerais was lower than expectations, with a rate of 7.3%, consistent with the findings of Parsani et al. (2013) in India and Canestrini-Trotti and Restani (1972) in Italy (Dubey and Bauer, 2018), which reported prevalence rates of approximately 6%. These results also align with the findings of Gundłach et al. (2004) in Poland. Despite the prevalence being lower than the estimated 13.04%, most properties did not show *E. leuckarti*, infection and where it was detected, risk factors associated with the infection were identified.

The analysis of foals indicated that, although they are more susceptible to *E. leuckarti* (Dubey and Bauer, 2018), there was no significant correlation between age and infection. Of the 247 foals, 10.5% of the younger ones and 4.5% of the older ones tested positive. This finding may be related to the observed prevalence and the study methodology, which distinguished between the nursing phase (0 to 6 months) and the weaned phase (7 to 12 months).

Dietary factors influenced the prevalence: the highest infection rates were observed in Stud Farm 3 (Ponte Nova) and 6 (Ubá), indicating a strong correlation between silage consumption and positivity ($p \leq 0.01$). Silage may affect gastrointestinal microbiota and favor the proliferation of *E. leuckarti* (Zhu et al., 2021). Out of 247 animals, 141 were fed silage, and 17 of the 18 positives were from Stud Farms 3 and 6. The intestinal microbiota plays a crucial role in immune modulation, preventing the colonization of pathogen, influencing mucosal immunity through mucus production, and activating immune cells. These processes, however, can be disrupted by abrupt dietary changes, which alter the balance of commensal microorganisms (Boucher et al., 2024).
Sugarcane, used in Stud Farms 2 (Guaraciaba) and 6 (Ubá), did not show an association with *E. leuckarti* infection. Despite being an alternative during dry periods, its high fiber content and sucrose concentration did not correlate with positivity. Neither chopped grass nor hay showed any relationship with infection.

No correlation was found between *Eimeria* infection and diarrhea, corroborating the absence of clinical signs in many cases (Gorji, Sadr, and Borji, 2023). Of the 247 animals evaluated, only 7 of the 63 with diarrhea tested positive.

Regarding fecal disposal of feces, although none of the correlations had statistically significant, pasture (RR=0.029) and hay (RR=0.14) showed risk ratios (RR) less than 1, suggesting a possible inverse association with eimeriosis. This may indicate that fecal management and environmental controls are important factors. The analysis of fecal disposal practices locations revealed that about one-third of eimeriosis cases could be attributed to pasture use, highlighting the importance of proper management.

Finally, no significant correlation was found between infection and housing conditions, such as stalls (p=0.061) and paddocks (p=0.983), since as several establishments used both types of facilities, allowing animals to come into contact with pasture.

5. Conclusion

The parasite *Eimeria leuckarti* was found in equids from the Zona da Mata region of Minas Gerais. This study found that foals fed with silage are more susceptible to *E. leuckarti* infection, possibly due to intestinal dysbiosis caused by the feed's fermentative

characteristics and resulting alterations in the microbiota. However, further studies are needed to characterize the role of silage in the equine intestinal microbiota and to understand how microbiota modulation influences the establishment of this coccidian.

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Briefing notes: CEUA Protocol: 50554278388; CEP protocol: 59014622.7.0000.5153.

6. References

AFONSO, Ana Lúcia Alves. **Parasitas intestinais e seu controlo numa população de equídeos estabulados na Escola das Armas em Mafra, Portugal.** 2016. Dissertação (Mestrado Integrado em Medicina Veterinária) — Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisboa, 2016.

BAUER, C. Prevalence of Eimeria leuckarti (Flesch, 1883) and intensity of faecal oocyst output in a herd of horses during a summer grazing season. **Veterinary Parasitology**, Amsterdam, v. 30, p. 11-15, 1988.

BOUCHER, Laurie et al. Current Understanding of Equine Gut Dysbiosis and Microbiota Manipulation Techniques: Comparison with Current Knowledge in Other Species. **Animals**, v. 14, n. 5, p. 758, 2024.

DUBEY, Jitender P.; BAUER, Christian. A review of Eimeria infections in horses and other equids. **Veterinary parasitology**, v. 256, p. 58-70, 2018.

GORJI, Faezeh Faghihzadeh; SADR, Soheil; BORJI, Hassan. Epidemiological study on equine coccidiosis in North and Northeast of Iran. **Veterinary Medicine and Science**, v. 9, n. 5, p. 2038-2041, 2023.

GUELPA, Gabriel Jabismar. **Detecção de Strongylus vulgaris por meio de técnica coproparasitológica em equinos naturalmente infectados**. 2023. Dissertação (Mestrado em Ciência e Tecnologia Animal) — Faculdade de Ciências Agrárias e Tecnológicas, Universidade Estadual Paulista, Dracena, 2023.

GUNDŁACH, J. L. et al. Parasites of the alimentary tract of horses from the Lublin district in the light of coproscopic and gross histopathological examinations. **Medycyna Weterynaryjna**, v.60. 2004.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA (IBGE**). Produção agropecuária: equinos.** Disponível em: https://www.ibge.gov.br/explica/producao-agropecuaria/equinos/br. Acesso em: 10 fev. 2025.

LIMA, X. M. T. de S. **Estudo do Complexo do Agronegócio Cavalo.** Brasília: Confederação da Agricultura e Pecuária do Brasil, 2016.

NASCIMENTO, A. J. S.; NARDI JUNIOR, G. A cultura equina e sua evolução. **Tekhne e Logos**, Botucatu, v. 12, n. 3, p. 37-48, dez. 2021.

PARSANI, Husen R. et al. Studies on gastrointestinal helminths of Equus acinus in North Gujarat, India. **Egyptian Journal of Biology**, v. 15, p. 13-20, 2013.

SHEATHER, A. Leslie. The detection of worm eggs in the fæces of animals, and some experiments in the treatment of parasitic gastritis in cattle. **Journal of Comparative Pathology and Therapeutics**, v. 36, p. 71-90, 1923.

TOMLJENOVIĆ, R.; BORANIĆ-ŽIVODER, S.; ČORAK, S. Horse riding tourism – definitional conundrum. In: 4th International Rural Tourism Congress, **Congress Proceedings**, 2018, p. 278-287.

ZHU, Yiping et al. Effects of pasture grass, silage, and hay diet on equine fecal microbiota. **Animals**, v. 11, n. 5, p. 1330, 2021.

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Clinical and behavioral markers of feather picking in trafficked Golden-capped parakeet (*Aratinga auricapillus*) in Brazil

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Abstract: Feather-damaging behavior (FDB) is a frequent condition in psittacines under human care, with implications for animal welfare and post-rehabilitation evaluation. This case study documents behavioral, clinical, hematological, and sex-related patterns in 10 *Aratinga auricapillus* individuals held at the Wild Animal Screening Center in Vitória da Conquista, Bahia, Brazil. Birds were housed under standardized conditions and assessed using feather condition scoring, molecular sexing, leukocyte profiling, pathogen screening, and open-field tests. Five individuals exhibited plumage damage consistent with self-inflicted feather picking, predominantly affecting the chest, back, and legs. All feather-damaged individuals were female; all males presented intact plumage. Microscopy confirmed structural feather degradation, and no ectoparasites or infectious agents were detected. Feather-damaged birds showed modestly higher heterophil-to-lymphocyte ratios, suggesting increased physiological stress. However, these differences were modest and did not show statistical support for an association with feather condition. Temperament profiles (shy vs. bold) were evenly distributed and showed no apparent relation to feather condition. These findings suggest a sex-biased expression of FDB in *A. auricapillus* and reinforce the utility of integrated clinical, behavioral, and physiological assessments in triage routines. Although preliminary, the present study provides baseline reference data for future evaluations of confiscated psittacines, offering practical guidance for rehabilitation and release programs, based on the presented index of feather degradation.
Keywords: behavior; biology; captive birds; hematology; psittacines; sex.

1. Introduction

Brazil harbors the second-highest global diversity of psittacine species (IUCN, 2024), many of which are threatened by habitat loss and the illegal wildlife trade. The golden-capped parakeet (*Aratinga auricapillus*), endemic to the Atlantic Forest, is among the psittacines most frequently confiscated by environmental authorities and is regularly admitted to wildlife rehabilitation centers across the country (Destro et al., 2012; Costa et al., 2018; Mendonça et al., 2020).

In Brazil, reintroduction into the wild is the standard institutional goal for seized wildlife, following clinical stabilization and behavioral assessment. This is particularly relevant for Centro de Triagem de Animais Silvestres (CETAS - Wild Animal Screening Center), which serves as a centralized hub for triage and temporary holding of trafficked animals. However, behavioral syndromes developed during captivity, such as stereotypies or self-directed behaviors, may compromise release potential. Among psittacines, psychogenic feather-damaging behavior (FDB), including feather picking and chewing, is one of the most common captivity-induced conditions, often associated with chronic stress, rearing methods, and environmental deprivation (Costa et al., 2016; Ebisawa et al., 2021; Ebisawa et al., 2022).

Understanding the behavioral and physiological profiles of individuals affected by FDB is essential for informing reintroduction decisions and improving animal welfare protocols in captivity. Yet most literature on FDB derives from long-term captives or companion birds, with limited data available for wild-caught individuals undergoing temporary rehabilitation (Acharya and Rault, 2020; Ebisawa et al., 2021; Mahdavi et al., 2023).

This study presents a case series of 10 *A. auricapillus* individuals held at a CETAS facility in Bahia, Brazil, focusing on plumage condition, hematological stress indicators, temperament profiles, and sex. We highlight patterns in the occurrence of FDB and discuss their relevance to rehabilitation management.

The specific objectives of this study were to: (i) assess the body feather condition score of the animals included in the study; (ii) perform molecular sexing of the birds; (iii) evaluate pathogens of significance for psittacines; (iv) determine stress levels through leukocyte-based indicators; and (v) conduct a behavioral assessment of the animals.

2. Materials e Methods

2.1. Study context and animal housing

This study was conducted at the CETAS in Vitória da Conquista, Bahia, Brazil, as part of a broader rehabilitation protocol for wild birds destined for reintroduction (Health and Behavioral Assessment of Wild Birds – Contributions to Reintroduction, protocol 093/2021, UFBA Animal Ethics Committee). Ten adult *A. auricapillus* individuals were selected among birds previously seized from wildlife trafficking or voluntarily surrendered by the public. Due to their origins, information on age, time in captivity, and rearing history was unavailable.

After routine quarantine and initial clinical screening, birds were housed communally in a 5 × 4 × 3 m enclosure, which was enriched weekly with fresh eucalyptus branches. They received water *ad libitum* and a diet of seeds and seasonal fruits. Identification was performed using a photographic catalog and non-toxic color markings on the chest region.

2.2. Clinical examination and categorization by feather condition

All individuals underwent complete physical examination with emphasis on plumage and integumentary integrity. Feathers from the pectoral region were collected and examined under stereomicroscopy (20×) to rule out ectoparasites and assess feather structure. Based on the visual presence or absence of feather loss, individuals were retrospectively categorized into two subsets for descriptive analysis:

- Feather-damaged group (n = 5): individuals presenting visible feather loss or damage consistent with feather picking, sometimes accompanied by superficial skin lesions.
- Intact-plumage group (n = 5): individuals with no visible feather loss or structural abnormalities.

This categorization was used solely for comparative purposes and did not reflect prior experimental assignment or treatment.

2.3. Feather condition scoring

Feather condition was scored using the 10-point system described by Meehan et al. (2003), which evaluates five body regions (chest/flank, back, legs, wings, tail). Each region received a score from 0 (severe feather loss) to 2 (intact), and scores were summed for each bird (maximum = 10). Standardized photographs were evaluated independently by three blinded observers.

2.4. Molecular sexing and pathogen screening

Blood samples were collected according to protocols described by Silva et al. (2021) and Fraga et al. (2023). Sex was determined by PCR amplification of the CHD gene (Fridolfsson and Ellegren, 1999; Vucicevic et al., 2012) using primers 2550F and 2718R, following the protocol adapted from Antonio et al. (2021).

To exclude non-psychogenic causes of feather loss, cloacal swabs were screened for *Chlamydia psittaci* and Beak and Feather Disease Virus (BFDV) using established PCR protocols (Hewinson et al., 1997; Ypelaar et al., 1999; Mirabal-Santos et al., 2023; Antonio et al., 2025).

2.5. Hematology and stress index

Blood smears were prepared for hematological profiling. Total and differential leukocyte counts were performed manually, along with thrombocyte counts. The heterophil-to-lymphocyte ratio (H:L) was calculated for each individual as an indirect indicator of physiological stress (Campbell, 2012; Davis et al., 2008). Reference values were drawn from Mello et al. (2016), Silva (2010), and Vaz et al. (2015).

2.6. Temperament evaluation

Temperament was assessed via a single-session open-field test following the procedures of Perals et al. (2017) and Silva et al. (2021). Each bird was filmed for 10 minutes in a novel environment after a 5-minute habituation period. Based on their behavior, birds were classified as:

- Bold: exited the transport cage and explored the arena.
- Shy: remained immobile or confined to the cage interior.

Latency to the first movement and the first step were recorded as secondary behavioral metrics.

2.7. Data Analysis

All data were treated descriptively due to the small sample size and observational design. Categorical variables (such as sex, feather condition, and temperament) were summarized using absolute frequencies. Continuous variables (such as feather scores, hematological values, and behavioral latencies) were described using summary statistics. Because the dataset fails to meet the core requirements for valid parametric inference, including normality, homoscedasticity, and independent random sampling, and presents severely unbalanced groups ($n \leq 5$), parametric tests would be statistically inappropriate and potentially misleading. To preserve rigor in the only planned comparison involving a continuous variable (heterophil-to-lymphocyte ratio across feather-condition groups), we used an exact Mann–Whitney U test. This nonparametric method is explicitly designed for small, non-normal samples and avoids inflated error rates that arise from forcing parametric models onto unsuitable data. Data processing and summarization were carried out in R version 4.1.0 (R Core Team, 2022).

3. Results

3.1. 3.1. Feather condition and clinical observations

Among the individuals evaluated, five presented visible feather loss, broken feathers, or localized skin lesions consistent with FDB. These birds were categorized as “feather-damaged” for comparative purposes. Feather damage predominantly affected the back, legs, chest, and tail, with feather fraying and worn barbs observed under stereomicroscopy. No ectoparasites or signs of pathogenic skin conditions were identified. The other five individuals exhibited intact plumage and no integumentary abnormalities (Figure 1).

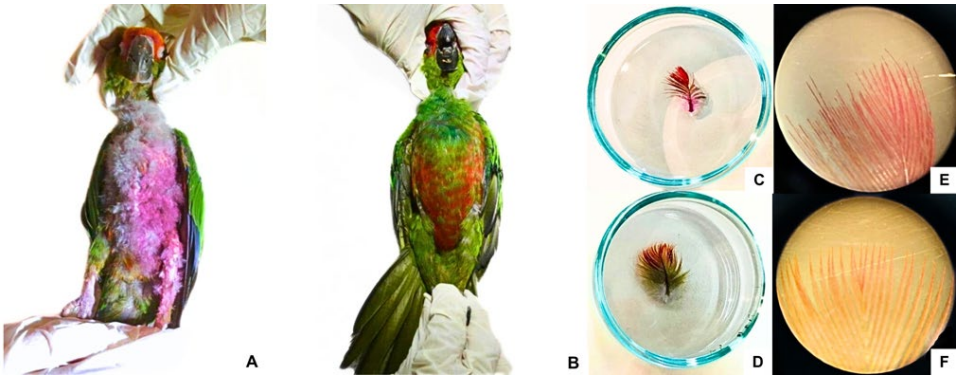

Figure 1 – (A, C, E) Feather-damaged individual: macroscopic view of ventral feather loss (a), isolated damaged feather (c), and frayed barbules under stereomicroscopy at 20× (e). (B, D, F) Individual with intact plumage: macroscopic view (b), undamaged feather (d), and organized barbules (f). Feather damage was associated with visible loss, frayed barbs, and structural disorganization.

Feather scoring revealed regional variation in the feather-damaged group, with the lowest scores in the back (mean 0.95 ± 0.41) and legs (1.00 ± 0.35), and relatively higher scores in the wings (1.8 ± 0.27). Total feather scores ranged from 4.5 to 6.25 in this group. All birds with intact plumage scored the maximum of 10. Examples of feather condition in affected (a–c) and unaffected (d–f) individuals are shown in Figure 2, with arrows indicating the anatomical regions used in the scoring system (chest/flank, wings, back, legs, tail).

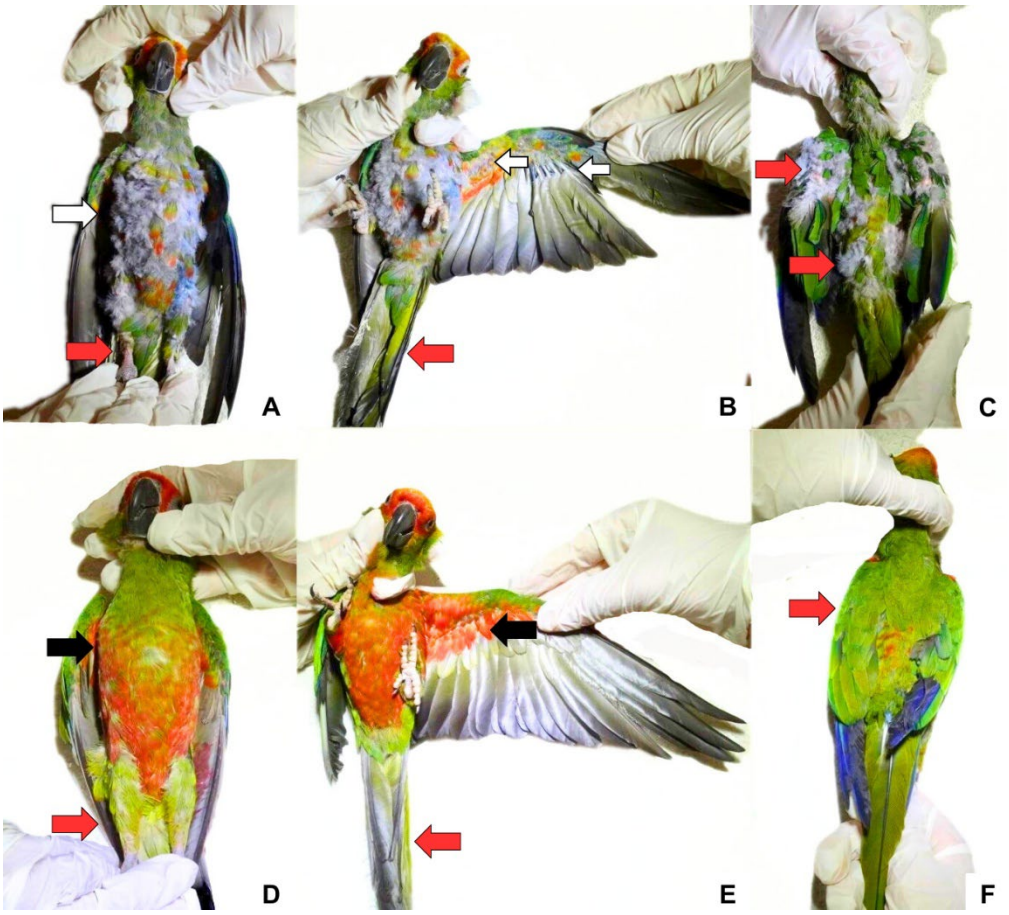

Figure 2 – Plumage condition in *Aratinga auricapillus* individuals with (A-C) and without (D-F) feather damage. Arrows indicate the five anatomical regions assessed in the feather condition scoring system: chest/flank, wings, back, legs, and tail (Meehan et al., 2003). Feather loss and structural disruption are visibly distributed across multiple regions in affected individuals (A-C), whereas plumage remains intact in unaffected individuals (D-F).

3.2. Sex distribution

Molecular sexing revealed that four of the five females in the sample were among the feather-damaged individuals. All birds with intact plumage were male. This complete overlap between sex and feather condition precluded statistical analysis of sex as an independent factor. However, the pattern observed is consistent with prior reports suggesting a higher prevalence of FDB in female psittacines (Ebisawa et al., 2022; Kinkaid et al., 2013).

3.3. Hematological profiles and stress indicators

Blood smears were successfully analyzed for nine of the ten birds. Mean leukocyte counts were slightly higher in feather-damaged birds (15.16 × 10³/µL) compared to birds with intact plumage (13.95 × 10³/µL). The mean heterophil-to-lymphocyte (H:L) ratio, an indirect indicator of chronic stress, was also elevated in the feather-damaged group (1.99 ± 0.20) relative to the intact group (1.69 ± 0.48). The exact Mann–Whitney U test did not detect a statistically significant difference between groups for the H:L ratio (two-tailed p = 0.25). However, values for all individuals fell within the general reference ranges reported for psittacine species (Mello et al., 2016; Silva, 2010; Vaz et al., 2015). These values are summarized in Table 1. A visual comparison of H:L ratio distributions by feather condition is presented in Figure 3. No hematological abnormalities or strong group-wise divergences were detected.

Hematological parameter	**Feather-damaged (n=5)** (mean±standard deviation)	**Intact plumage (n=4)** (mean±standard deviation)
Total Leukocytes	15.16±1.84 (x10³/µL)	13.95±0.95 (x10³/µL)
Monocytes	0.98±0.20 (x10³/µL)	0.94±0.33 (x10³/µL)
Heterophils	8.83±0.89 (x10³/µL)	7.70±0.66 (x10³/µL)
Lymphocytes	4.44±0.72 (x10³/µL)	4.69±0.90 (x10³/µL)
Eosinophils	0.89±0.34 (x10³/µL)	0.59±0.19 (x10³/µL)
Basophils	0.00±0.00 (x10³/µL)	0.00±0.00 (x10³/µL)
Heterophil:lymphocyte ratio (H:L)	1.99±0.20	1.69±0.48
Thrombocyte	15.12±1.06 (x10³/µL)	15.40±1.27 (x10³/µL)

Table 1 – Hematological parameters in *Aratinga auricapillus* individuals with and without feather damage. Values are presented as mean ± standard deviation. All counts expressed in ×10³/µL, except H:L ratio (unitless). Blood smear data were unavailable for one individual with intact plumage.
Note: All values expressed in ×10³/µL, except for the H:L ratio (unitless). Smear data missing for one individual with intact plumage.

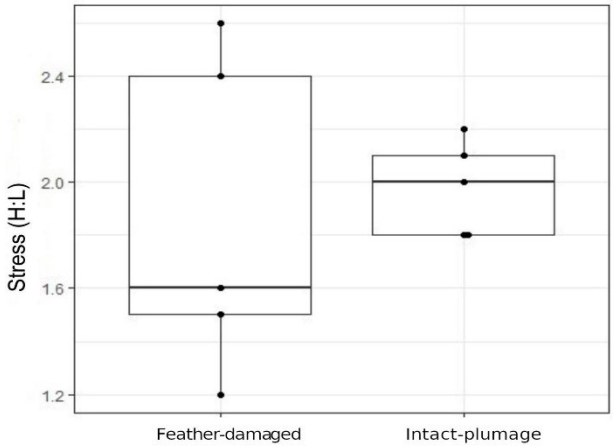

Figure 3 – Heterophil-to-lymphocyte (H:L) ratio in *Aratinga auricapillus* individuals categorized by feather condition. Boxplots display the distribution of values for each group (n=5 feather-damaged, n=4 intact-plumage). No statistical tests were performed; overlap between groups suggests only modest differences.

3.4. Temperament profiles

Based on the open field test, six birds were classified as shy and four as bold. The distribution of temperament types was the same across plumage categories, with three shy and two bold individuals in each group. Shy birds exhibited longer latencies to initiate movement and more prolonged freezing behavior, consistent with the behavioral criteria used for classification. No apparent association was observed between temperament type and feather condition in this sample.

4. Discussion

FDB in psittacines under human care is well-documented and is typically associated with chronic stress, inadequate environmental stimulation and rearing methods, or underlying physiological imbalances (Costa et al., 2016; Ebisawa et al., 2021; Ebisawa et al., 2022). In this study, feather loss and structural feather damage were observed in half of the sampled *A. auricapillus* individuals housed at a CETAS facility. Microscopic feather analysis, clinical inspection, and pathogen screening supported the diagnosis of psychogenic feather picking rather than an infectious or parasitic origin.

The most striking pattern observed was that all individuals with feather damage were female. While this is consistent with previous findings in other psittacine species, in which females have shown a higher incidence of FDB (Ebisawa et al., 2022; Kinkaid et al., 2013), the complete overlap between sex and feather condition in our sample precludes any conclusion regarding causality. It

remains unclear whether the pattern reflects biological predisposition, housing history, or sampling artifact. Larger and sex-balanced datasets will be necessary to assess the role of sex more rigorously.

Physiological stress, as estimated by leukocyte profiles and the heterophil-to-lymphocyte (H:L) ratio, was slightly elevated in birds with feather damage. However, these differences were modest, fell within psittacine reference ranges (Mello et al., 2016; Silva, 2010), and did not show statistical support for an association with feather condition, given the sample size and sex collinearity. Moreover, because H:L ratios are sensitive to handling and baseline fluctuations, they may be insufficient to capture stress dynamics in rehabilitation settings. Complementary markers, such as fecal glucocorticoid metabolites, could provide more robust assessments (Davis and Maney, 2018; Grundei et al., 2024), though they were beyond the scope of this study.

Temperament evaluation via the open field test identified both shy and bold individuals, but no apparent association was found between behavioral profile and feather condition. This result should be interpreted cautiously, as the sample size was small and temperament was assessed through a single exposure. Nevertheless, the use of temperament profiling remains relevant to rehabilitation planning, particularly given its potential influence on post-release behavior and stress-coping capacity (Ramos et al., 2021; Silva et al., 2021).

Importantly, all birds in this study were part of a broader institutional program to evaluate health and behavior before reintroduction. FDB, by compromising thermoregulation and flight ability, poses a significant obstacle to release and long-term survival (Brando and Norman, 2023; Pough et al., 2013). Understanding the behavioral and physiological profiles of seized individuals is therefore essential not only for individual welfare but also for the success of conservation translocation programs.

5. Conclusion

The results of this study are preliminary, indicating a sex-biased expression of FDB in *A. auricapillus*. The data highlight the importance of integrated behavioral and physiological assessments of birds during triage routines. While the present findings are limited by a small sample size and categorical overlap among key variables, they offer preliminary insights into the occurrence and correlates of FDB in *A. auricapillus* during rehabilitation.

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6. References

Acharya, R.; Rault, J. L. Risk factors for feather-damaging behavior in companion parrots: a social media study. J Vet Behav, v. 40, p. 43–49, 2020.

Antonio, E. S.; Sacramento-Batista, P.; Teixeira, S. B.; Barros, J. S.; Gobbo, G. D.; Fraga, R. E.; Tomazi, L. Levantamento da proporção sexual em *Ara chloropterus* Gray, 1859 em CETAS da Bahia, Brasil. Rev Bras Ciênc, v. 28, p. 218–224, 2021.

Antonio, E. S.; Fraga, R. E.; Sacramento, P.; Freitas, A. L. D.; Santana, A. C. B.; Barbosa, S.; Dominato, R. C.; Silva, J. G. Molecular assessment of *Chlamydia psittaci* and *Circovirus* in psittacines from a CETAS in Bahia, Brazil. Brazilian Journal of Microbiology, v. 56, p. 927–938, 2025.

Brando, S.; Norman, M. Handling and Training of Wild Animals: Evidence and Ethics-Based Approaches and Best Practices in the Modern Zoo. Animals, v. 13, p. 2247, 2023.

Campbell, T. W. Hematology of Birds. In: Thrall, M. A.; Weiser, G.; Allison, R. W.; Campbell, T. W. (eds.) Vet Hematol Clin Chem, 2nd ed., Wiley-Blackwell, Hoboken, p. 507–593, 2012.

Costa, P.; Macchi, E.; Valle, E.; Marco, M.; Nucera, D. M.; Gasco, L.; Schiavone, A. An association between feather damaging behavior and corticosterone metabolite excretion in captive African grey parrots (*Psittacus erithacus*). PeerJ, v. 4, e2462, 2016.

Costa, F. J. V.; Ribeiro, R. E.; de Souza, C. A.; Navarro, R. D. Espécies de aves traficadas no Brasil: uma meta-análise com ênfase nas espécies ameaçadas. Fronteira: Journal of Social, Technological and Environmental Science, v. 7, n. 2, p. 324-346, 2018.

Davis, A. K.; Maney, D. L. The use of glucocorticoid hormones or leucocyte profiles to measure stress in vertebrates: What's the difference? Methods Ecol Evol, v. 9, p. 1556–1568, 2018.

Davis, A. K.; Maney, D. L.; Maerz, J. C. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Funct Ecol, v. 22, p. 760–772, 2008.

Destro, G. F. G.; Pimentel, T. L.; Sabaini, R. M.; Borges, R. C.; Barreto, R. Efforts to Combat Wild Animals Trafficking in Brazil. In: Lameed, G. A. (ed.) Biodivers Enrich Divers World. InTechOpen, London, p. 421–436, 2012.

Ebisawa, K.; Nakayama, S.; Pai, C.; Kinoshita, R.; Koie, H. Prevalence and risk factors for feather-damaging behavior in psittacine birds: Analysis of a Japanese nationwide survey. PloS one, v. 16, n. 7, p. e0254610, 2021.

Ebisawa, K.; Kusuda, S.; Nakayama, S.; Pai, C.; Kinoshita, R.; Koie, Hl. Effects of rearing methods on feather-damaging behavior and corticosterone metabolite excretion in the peach-faced lovebird (*Agapornis roseicollis* Vieillot). Journal of Veterinary Behavior, v. 54, p. 28–35, 2022.

Fraga, R. E.; Santos, C. M. R.; Silva, R. S.; Teixeira, R. E. R.; Tomazi, L.; Silva, M. B.; Zapelini, C.; Schiavetti, A. Reintroduction and monitoring of the bird Amazona aestiva (Psittaciformes: Psittacidae) in Brazil. Rev Biol Trop, v. 71, e53145, 2023.

Fridolfsson, A. K.; Ellegren, H. A simple and universal method for molecular sexing of non-ratite birds. J Avian Biol, v. 30, p. 116–121, 1999.

Grundei, L. L.; Wolf, T. E.; Brandes, F.; Schütte, K.; Freise, F.; Siebert, U.; Touma, C.; Pees, M. Validation of fecal glucocorticoid metabolites as non-invasive markers for

monitoring stress in common buzzards (*Buteo buteo*). Animals, v. 14, n. 8, p. 1234, 2024.

Hewinson, R. G.; Griffiths, P. C.; Bevan, B. J.; Kirwan, S. E. S.; Field, M. E.; Woodward, M. J.; Dawson, M. Detection of Chlamydia psittaci DNA in avian clinical samples by polymerase chain reaction. Vet Microbiol, v. 54, p. 155–166, 1997.

IUCN. The IUCN Red List of Threatened Species, version 2024-1. Disponível em: https://www.iucnredlist.org. Acesso em: 15 ago. 2024.

Kinkaid, H. Y. M.; Mills, D. S.; Nichols, S. G.; Meagher, R. K.; Mason, G. J. Feather-damaging behaviour in companion parrots: an initial analysis of potential demographic risk factors. Avian Biol Res, v. 6, p. 289–296, 2013.

Mahdavi, I.; Abdi-Hachesoo, B.; Ansari-Lari, M.; Haddad-Marandi, M. R. Prevalence and risk factors of feather damaging behavior in companion parrots: a cross-sectional study in Iran. Appl Anim Behav Sci, v. 266, p. 106028, 2023.

Meehan, C. L.; Millam, J. R.; Mench, J. A. Foraging opportunity and increased physical complexity both prevent and reduce psychogenic feather picking by young Amazon parrots. Appl Anim Behav Sci, v. 80, p. 71–85, 2003.

Mello, R. H.; Martins, R. F.; Bonissi, D. A.; Junior, J. L. B.; Melotti, T.; Bianchi, H.; Bianchi, M. A. F. Haematological reference for red-browed parrot (Amazona rhodocorytha, Salvadori, 1890) captive in the Atlantic Forest in Eastern Brazil. Arq Bras Med Vet Zootec, v. 68, p. 1275–1282, 2016.

Mendonça, R.; Amaral, A. A. N.; Voltolini, J. C. Recepção, triagem e soltura de psitacídeos no Centro de Triagem de Animais Silvestres (CETAS) IBAMA Lorena, SP. Revista Biociências, v. 26, n. 1, p. 70-79, 2020.

Mirabal-Santos, B.; Antonio, E. S.; Pereira, D. C.; Dourado, A. T. T.; Silva, M. B.; Fraga, R. E.; Tomazi, L. Determining the prevalence of avian chlamydiosis in wild Amazona species from Brazil using molecular testing and clinical signs. J Avian Med Surg, v. 37, p. 32–40, 2023.

Perals, D.; Griffin, A. S.; Bartomeus, I.; Sol, D. Revisiting the open-field test: what does it really tell us about animal personality? Anim Behav, v. 123, p. 69–79, 2017.

Pough, F. H.; Bemis, W. E.; McGuire, B. A.; Janis, C. M. Vertebrate Life, 11th ed. Oxford: Oxford University Press, 2022.

R Core Team. R: A language and environment for statistical computing. Disponível em: https://www.r-project.org. 2022.

Ramos, G. D. A.; Azevedo, C. S. D.; Jardim, T. H.; Sant'Anna, A. C. Temperament in captivity, environmental enrichment, flight ability, and response to humans in an endangered parrot species. Journal of Applied Animal Welfare Science, v. 24, n. 4, p. 379–391, 2021.

Silva, G. F. N. Perfil hematológico de psitacídeos mantidos em cativeiro. Dissertação, Universidade Federal do Vale do São Francisco, 2010.

Silva, R. S.; Fraga, R. E.; Tomazi, L.; Andrade, T. S.; Silva, M. S.; Schiavetti, A. Temperament assessment and pre-release training in a reintroduction program for the Turquoise-fronted Amazon *Amazona aestiva*. Acta Ornithol, v. 55, p. 199–214, 2021.

Vaz, F. F.; Locatelli-Dittrich, R.; Sipinski, E. A. B.; Abbud, M. C.; Sezerban, R. M.; Schmidt, E. M. S.; Dittrich, J.; Cavaleiro, M. L. Hematologic and total plasma protein values in free-living red-tailed Amazon parrot nestlings (Amazona brasiliensis) in Parana State, Brazil. J Avian Med Surg, v. 29, p. 187–191, 2015.

Vucicevic, M.; Stevanov-Pavlovic, M.; Stevanovic, J.; Bosnjak, J.; Gajic, B.; Aleksic, N.; Stanimirovic, Z. Sex determination in 58 bird species and evaluation of CHD gene as a universal molecular marker in bird sexing. Zoo Biol, v. 32, p. 269–276, 2012.

Ypelaar, I.; Bassami, M. R.; Wilcox, G. E.; Raidal, S. R. A universal polymerase chain reaction for the detection of psittacine beak and feather disease virus. Vet Microbiol, v. 68, p. 141–148, 1999.

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The incidence of pododermatitis on broiler carcasses: lesion characteristics and sensitivity of acetic acid on associated bacteria

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Abstract: Broiler chicken feet are used as food and in the production of gelatin in many countries. Pododermatitis is a common disease in broiler chicken farming, which can affect the quality of broiler feet and be a perennial source of pathogenic bacteria that threaten public health. This research aimed to describe the lesion characteristics (macroscopic and histopathological) of pododermatitis in slaughtered broiler chickens, identify the associated pathogenic bacteria, and evaluate their sensitivity to acetic acid. One hundred thirty-six samples of pododermatitis lesions were subjected to morphological examination, and 129 underwent bacteriological examination to isolate and identify pathogenic bacteria using standard microbiological and biochemical tests. All isolated bacterial strains were tested for sensitivity to acetic acid using the agar dilution method. Pododermatitis samples of different scores underwent histopathological analysis. The results showed that score 4 was the most common (37.5%) for pododermatitis with an area of 2.91±2.00 cm^2. Pododermatitis scores 3 and 4 showed the same microscopic lesions, including full-thickness epidermal ulcerations. Bacteriological analysis revealed that all 129 pododermatitis samples were positive, and 184 bacterial strains were isolated and identified. *Staphylococcus aureus* was the most frequently isolated bacterium (66.30%), and the second- and third-most prevalent were *Escherichia coli* (19.57%) and *Staphylococcus hiycus* (5.98%), respectively. Acetic acid was particularly effective against all tested bacterial strains (*Staphylococcus aureus*, *Staphylococcus hiycus*, *Escherichia coli*, *Salmonella enterica*, *Shigella flexneri,* and *Shigella sonnei*), with an average minimum inhibitory concentration (MIC) of ≤0.08%. In conclusion, broiler pododermatitis was a dominant disease, and *S. aureus* was the most important pathogen associated with pododermatitis in broiler chickens. Acetic acid was an effective product for controlling the bacteria involved in pododermatitis.

Keywords: Pododermatitis, lesions, incidence, broiler chickens, bacteria, acetic acid.

1. Introduction

In recent years, the poultry industry has experienced remarkable growth, resulting in a significant increase in the production of broilers and white meat at affordable costs for consumers. The marketing and use of broiler feet in human food are widespread practices in many cultures around the world. As a source of protein, chicken feet are also prized for their nutritional value (Taufik et al., 2010). However, it is essential to emphasize the importance of decontaminating the feet to ensure their safety. Consumers are becoming increasingly aware of these issues, thus favoring a more responsible approach to the marketing and use of chicken feet. Chicken feet are also used as raw material in the production of gelatin (Tiona and Rahelivolololoniaina, 2023). Gelatin is widely used in the food industry to thicken, gel, and stabilize various products such as confectionery, desserts, yogurts, and even some savory dishes. This use of chicken feet helps minimize waste by efficiently harnessing animal resources to produce versatile ingredients in various culinary and industrial applications.

Pododermatitis, also known as foot pad dermatitis, is a disease characterized by necrotic lesions on the plantar surface of the foot pads of growing broilers (Jacob et al., 2016). Symptoms of pododermatitis include hard, scaly foot pad skin, abnormal keratin horn-like ankles, swollen and frequently splitting foot pad, and necrotic lesions of the epidermis that often split down the center of the lesion (Mayne, 2005). This disease is prevalent in broiler farms and has been reported in several countries. It poses a challenge to the modern poultry industry, as it is one of the most detrimental locomotor disorders affecting broiler welfare and a serious economic problem for broiler production (Szafraniec et al., 2022). Pododermatitis also alters behavior and serves as a port of entry for pathogens, especially litter bacteria, which are in constant contact with the footpads. Many factors can contribute to the development of pododermatitis, including broiler feed, skin structure, body weight, sex, humidity, and litter type. However, wet bedding is the factor most likely to affect pododermatitis (Mayne, 2005).

Acetic acid is a well-established agent as an antiseptic, disinfectant, and food preservative, long recognized for its antimicrobial potential. Its use in controlling bacteria associated with pododermatitis in broiler chickens, whether on farms, in poultry slaughterhouses, or at chicken foot processing facilities, helps minimize the risk of infection for both poultry and consumers. To date, knowledge of broiler pododermatitis characteristics is limited. Therefore, the objective of this study is to fill this gap by investigating the lesion characteristics of pododermatitis in slaughtered broilers and determining the pathogenic bacteria present in these lesions and their sensitivity to acetic acid.

2. Materials e Methods

a. Ethical approval

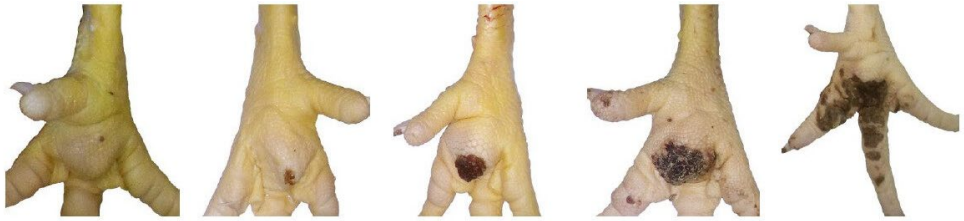
No ethical approval was obtained because this study did not involve laboratory animals and only involved non-invasive procedures.

b. Chicken selection and sampling

This study was conducted in two poultry slaughterhouses in Algeria. The samples were collected from 29 farms distributed across the eastern region of the country. Broilers were reared in buildings equipped with wood chip bedding. All broilers, of mixed sexes, were fed Algerian commercial feed and a standard fattening diet (starter, grower and finisher), and vaccinated according to a standard vaccination protocol. They were slaughtered on the 45th day. A total of 136 broilers with pododermatitis were randomly selected after slaughter. The carcasses were obtained at the *post-mortem* inspection line, and the legs were then examined, swabbed, and photographed using a digital camera. Each sample consisted of a single leg per bird. This procedure made it possible to collect data on the lesional and bacteriological characteristics of these foot lesions.

c. Visual annotation of pododermatitis

Affected legs were visually assessed and classified for pododermatitis by one person, according to a visual scoring system described by Welfare Quality (2009). Based on personal observations, macroscopic scores were assigned to each lesion to assess its severity, using the lesion characteristics reported by Piller et al. (2020) for pododermatitis. The scoring scale has five levels (0, 1, 2, 3, and 4) and is represented by photos (Figure 1). Table 1 shows the lesion characteristics of each pododermatitis score, according to the report by Piller et al. (2020).



Score 0 **Score 1** **Score 2** **Score 3** **Score 4**

Figure 1 – Visual scoring scale for evaluating pododermatitis, recommended by Welfare Quality (2009).

Score	Lesion characteristics
0	Physiological, non-lesional skin
1	Single superficial* lesion or several cumulative superficial* lesions or deep lesions** ≤ 0.5 cm
2	Superficial* lesion > 0.5 cm or deep** lesion > 0.5 cm to ≤ 1.0 cm
3	Deep** lesion > 1.0 cm
4	Lesion on the footpad and one or more deep lesions** on the toe

*: Lesions with discoloration, hyperkeratosis, no visual disturbance of epidermal layers; **: Lesions with loss of scales, visual disturbance of epidermal layers

Table 1 – Characteristics of the lesions corresponding to each pododermatitis score, according to Piller et al. (2020).

d. Macroscopic measurements of pododermatitis

Photographs of affected legs were used to macroscopically measure the size of each pododermatitis lesion using ImageJ software (*ImageJ 1.54d*; Java *1.8.0_345, 64-bit*).

e. Histopathological analysis of pododermatitis

Fifty samples of pododermatitis lesions were collected (10 samples for each score) and placed in vials containing 10% formalin for histopathological analysis. Fixed tissues were dehydrated, cleared, and embedded in paraffin, and 5 µm histological sections were prepared and stained with Hematoxylin and Eosin (H&E) according to standard histological methods. Microscopic examination was performed using an Euromex® microscope equipped with a camera and Image Focus Plus V2 software, at different magnifications to detect microscopic lesions in the epidermis and dermis of the foot pad.

f. Bacteriological analysis of pododermatitis

A total of 129 samples of pododermatitis lesions collected from two poultry slaughterhouses were submitted for bacteriological analysis, excluding seven small pododermatitis lesions (score 1) that were not suitable for swabbing. The pododermatitis lesion samples had all different scores. Bacteriological analysis was carried out within 24 h of collection. The swabs were pre-enriched for 24 h at 37 °C in Brain Heart Infusion (BHI) (BIOKAR®, France). Then, 10 µL of each turbid broth was directly plated onto Chapman agar (BIOKAR®, France) and Hektoen agar (BIOKAR®, France), and cultured aerobically at 37 °C for 24-48 h to detect contamination with staphylococci and enterobacteria, respectively. Salmonella detection was carried out according to the ISO 6579-1:2017 method. The different colonies were subcultured on the same culture media for purification, and the bacterial strains were identified using standard microbiological and biochemical tests with API Staph and API 20E test strips (BioMérieux®, France) (Markey et al., 2013).

g. Determination of the MIC of acetic acid

All bacterial strains were subjected to an agar dilution test to determine the minimum inhibitory concentration (MIC) of acetic acid. This test was performed in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI,

2018). Muller-Hinton agar (BIOKAR®, France) was used for this protocol, with acetic acid concentrations adjusted to 2.5%, 1.25%, 0.63%, 0.31%, 0.16% and 0.08% (V/V). The agar was then inoculated with a standardized bacterial suspension at a density corresponding to the McFarland 0.5 standard (approximately 10^8 CFU/mL). This suspension was diluted to 10^7 CFU/mL, and 2 µL of this dilution was spotted on the agar plates, corresponding to approximately 10^4 CFU per spot. An agar plate without acetic acid served as a control. After aerobic incubation at 37 °C for 24 h, the plates were visually inspected for bacterial growth. The absence of growth indicated sensitivity to the tested acetic acid concentration, while the presence of bacterial colonies indicated resistance.

3. Results

a. Scoring and morphometry of pododermatitis lesions

During the visual and morphometric evaluation of pododermatitis, it was observed that score 4 was the most frequent (51/136; 37.5%) with an average area of 2.91±2.00 cm^2, followed by score 2 (42/136; 30.88%) with an average area of 0.5±0.18 cm^2, score 3 (23/136; 16.91%) with an average area of 1.29±0.79 cm^2, while score 1 was the least frequent (20/136; 14.71%) with an average area of 0.15±0.08 cm^2. The rates and areas of each pododermatitis score are shown in Table 2.

Lesion score	Number and rate of affected legs	Lesion area (mean ± standard deviation)
Score 1	20/136 (14.71%)	0.15±0.08 cm^2
Score 2	42/136 (30.88%)	0.5±0.18 cm^2
Score 3	23/136 (16.91%)	1.29±0.79 cm^2
Score 4	51/136 (37.5%)	2.91±2.00 cm^2

Table 2 – Prevalence and morphometry of the different pododermatitis scores.

b. Histopathology of pododermatitis

Concerning histopathological analysis of pododermatitis, score zero corresponded to a healthy skin structure, without histological abnormalities, with regular thickness of the keratin, epidermis, and dermis layers. Score 1 was characterized by mild epidermal hyperkeratosis and hyperplasia, as well as superficial dermal edema, congestion, and mild inflammatory infiltration. Score 2 showed marked hyperkeratosis and hyperplasia of the epidermis, accompanied by areas of inflammation with loss of epidermis in parts of the lesion, as well as superficial dermal congestion and inflammatory infiltration. Score 3 was mainly characterized by ulceration, with full-thickness necrosis of the epidermis replaced by necrotic and suppurative material, as well as marked hyperkeratosis, loss of epidermis, and an area of diffuse inflammation in the dermis. Score 4 corresponded to microscopic lesions that were seen in score 3. Figure 2 presents the different histological characteristics associated with each pododermatitis score in broiler chickens.

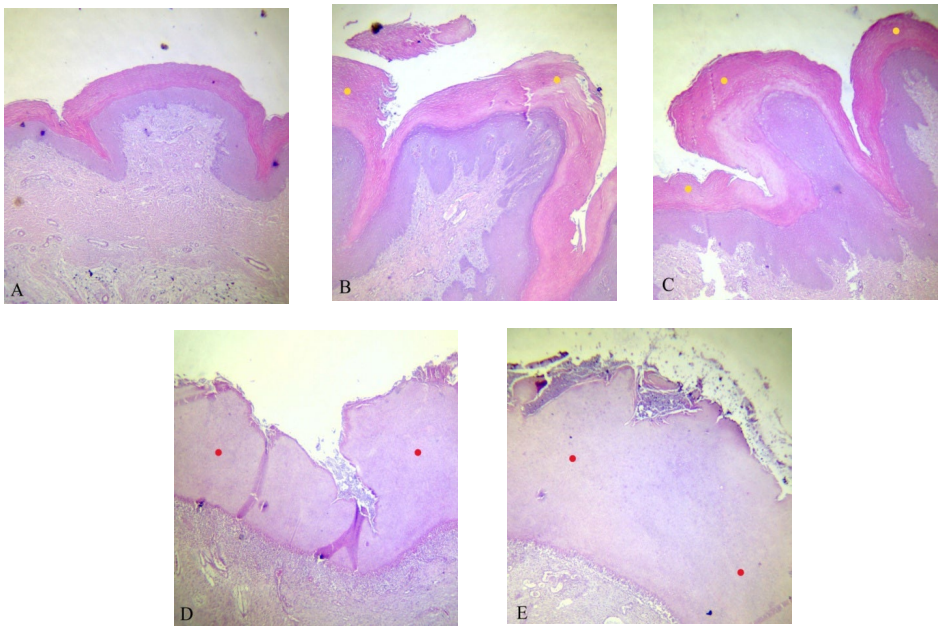


Figure 2 – Histological characteristics of different pododermatitis lesion scores (A: score 0, B: score 1, C: score 2, D: score 3 and E: score 4). Hyperkeratosis (yellow point); necrosis (red point) (H&E staining, 40X).

c. Bacteriology of pododermatitis

All 129 samples taken for bacteriological analysis of pododermatitis were positive, and allowed the isolation of 184 bacterial strains belonging to six different species. Of these samples, the majority (91/129; 70.54%) had infection with a single bacterial

species, while 26/129 samples (20.16%) had co-infection with two bacterial species, 9/129 samples (6.98%) a co-infection with three bacterial species, 2/129 samples (1.55%) a co-infection with four bacterial species, and 1/129 sample (0.77%) a co-infection with five bacterial species. Table 3 presents the number and rate of samples with co-infections.

Number of bacterial species of co-infection	Number and rate of samples
1	91/129 (70.54%)
2	26/129 (20.16%)
3	9/129 (6.98%)
4	2/129 (1.55%)
5	1/129 (0.77%)

Table 3 – Prevalence of bacterial co-infections in pododermatitis lesions.

The isolated bacteria belong to two bacterial families, with a higher rate among staphylococci (133/184; 72.28%) than among enterobacteria (51/184; 27.72%). The most frequently isolated bacterial strain was *Staphylococcus aureus*, representing 122/184 isolates (66.30%), followed by *Escherichia coli*, with 36/184 isolates (19.57%). The other bacterial species, such as *Staphylococcus hyicus*, *Shigella flexneri*, *Shigella sonnei,* and *Salmonella enterica,* were isolated at lower rates, with 11/184 isolates (5.98%), 8/184 isolates (4.35%), 4/184 isolates (2.17%), and 3/184 isolates (1.63%), respectively. Table 4 summarizes the number and rate of bacterial species isolated from 129 pododermatitis lesions.

	Bacteria	Number	Rate (%)
Staphylococci	*Staphylococcus aureus*	122/184	66.30%
	Staphylococcus hyicus	11/184	5.98%
	Total	133/184	72.28%
Enterobacteria	*Escherichia coli*	36/184	19.57%
	Salmonella enterica	3/184	1.63%
	Shigella flexneri	8/184	4.35%
	Shigella sonnei	4/184	2.17%
	Total	51/184	27.72%

Table 4 – Prevalence of bacterial species isolated from pododermatitis samples in 129 chickens.

d. MIC of acetic acid

The MIC of acetic acid was evaluated, and all bacterial strains had a MIC ≤ 0.08%.

4. Discussion

Pathogenic bacteria associated with pododermatitis have not received much attention. However, these pathogenic bacteria can induce other types of infections in chickens with pododermatitis or be transmitted to consumers of white meat by the food chain (Thøfner et al., 2019). The in-depth study of pododermatitis in broilers and associated bacteria is crucial not only for animal welfare but also for the quality of broiler feet for human consumption. The lesional characteristics of pododermatitis may directly affect the quality of chicken feet as food and as a raw material for gelatin. Skin lesions can alter the texture, appearance, and wholesomeness of chicken feet, thereby influencing their attractiveness in the food market. Furthermore, the presence of bacteria associated with pododermatitis underscores the importance of maintaining strict health standards in broiler foot production to protect public health. By understanding these aspects, researchers can help develop breeding practices and processing procedures that preserve the quality of broiler feet as a food and raw material, thereby ensuring food safety and consumer satisfaction.

According to Hashimoto et al. (2013), there is a positive correlation between the severity of pododermatitis and the condemnation rate, while a negative correlation was observed between this disease and live weight and thigh meat yields. Therefore, controlling pododermatitis could play a vital role in reducing condemnations and improving broiler performance.

In a study conducted by Dinev et al. (2019) in Bulgaria, the prevalence of contact dermatitis lesions in broilers was 21.87%. Pododermatitis was the most common lesion, affecting 15.7% of chickens (Dinev et al., 2019). In another study conducted in Japan on 45 flocks of broilers, all the birds examined had lesions of pododermatitis. The incidence of pododermatitis ranged from 31.9% to 99.5% across flocks, with lesion scores ranging from 0 to 3. Lesion scores affected 13.1, 33.3, 33.4, and 20.2% of broilers, respectively (Hashimoto et al., 2011). The study by Martrenchar et al. (2002) showed that only 10% of broiler flocks were of high quality, with 80% of birds scoring 0 for pododermatitis lesions. In addition, de Jong et al. (2012) reported that in 386 Dutch flocks, 35.5% of broilers showed no lesions, while 26.1% and 38.4% showed mild or severe lesions, respectively, and the severity of pododermatitis decreased with age.

Our study revealed histopathological lesions with different pododermatitis scores, similar to those reported by Piller et al. (2020), thereby corroborating our results. In our study, the scores for lesions 3 and 4 were similar and, apparently, caused by a pathogen-driven inflammatory process, which poses a risk to public health. Furthermore, our results corroborate those of Martins et al. (2016), who reported that higher scores are associated with more severe dermal and epidermal lesions. Additionally, our study showed that increases in the macroscopic score are associated with greater severity of microscopic lesions and deeper inflammation, as also reported by Piller et al. (2020).

The results of our study on the identification of bacteria isolated from pododermatitis agree with those reported by several authors, who also isolated the same bacterial species (*S. aureus*). Youssef et al. (2019) reported that *S. aureus* was isolated from 45.8% of affected chickens, either in pure form (18.18%) or in mixed culture with other bacterial species, including *E. coli* (58.18%), *Proteus mirabilis* (14.67%), and *Pseudomonas aeruginosa* (9.1%). Olsen et al. (2018) identified bacteria from pododermatitis lesions in laying hens, with a predominance of *S. aureus* (68%), followed by *Enterococcus faecalis* (14%), *E. coli* (9%), *S. hyicus* (4.5%), *Gallibacterium anatis* (2.7%), *Trueperella pyogenes* (1.8%), and *Aerococcus urinaeequi* (0.9%). In another study, Chaudhary et al. (2018) found that pododermatitis due to *S. aureus* was present in 89.65% of laying hens, 80.64% of broilers, and89.28% of backyard poultry.

The results of our study highlight the frequent isolation of *S. aureus*, *E. coli,* and, to a lesser extent, *S. enterica* from pododermatitis lesions in broiler chickens. These bacterial species are known to cause food poisoning in humans. It is therefore essential that veterinary practitioners and poultry slaughterhouse inspectors pay particular attention to this condition and take the necessary precautions to control these pathogenic bacteria and protect public health. Pododermatitis lesions can serve as sources of pathogenic bacteria in poultry slaughterhouses. During the slaughter of chickens, these bacteria can spread through the environment and contaminate chicken meat. These pathogenic bacteria can then cause infections in humans, either through direct contact or by consuming contaminated food or meat.

As for the MIC of acetic acid, it was found to be <0.08% for almost all bacterial strains. These results are encouraging and corroborate previous research. Derbal and Hanfer (2024) also demonstrated that the MIC of acetic acid was 0.08% against isolates of *S. aureus* and *E. coli* from the environment of poultry slaughterhouses, as well as from the carcasses and offal of broiler chickens. Fraise et al. (2013) demonstrated that acetic acid was effective at concentrations as low as 0.166% against various bacterial pathogens. Furthermore, Amrutha et al. (2017) found MICs of 1.5% for *E. coli* and 1% for *Salmonella* sp. Ouattara et al. (1997) also reported that acetic acid concentrations ranging from 0.1% to 1% (W/V) inhibited the growth of several bacteria responsible for meat spoilage. These results confirm that acetic acid is a potent and effective inhibitor of pathogenic bacteria.

Various studies have demonstrated the antibacterial effectiveness of acetic acid in reducing bacterial load on broiler carcasses. These studies have focused on a diverse range of bacteria, including mesophiles, coliforms, *E. coli,* and *S. aureus* (Abdul Wahid, 2008), *C. jejuni* (Zhao and Doyle, 2006), and *S. typhimurium* (Tamblyn and Conner, 1997). Furthermore, Ouattara et al. (1997) demonstrated that acetic acid completely inhibits the growth of bacteria responsible for meat spoilage. Therefore, the application of acetic acid to decontaminate feet affected by pododermatitis could significantly reduce the microbial load in the environment of poultry slaughterhouses, thereby favoring the production of salubrious broiler feet.

Decontamination of feet affected by pododermatitis should help reduce microbial biofilms in a meat processing environment (Ducková et al., 2023). Decontamination of feet affected by pododermatitis should be added as a food safety management choice. Prerequisite programs, such as good hygienic practice (GHP)/good manufacturing practice (GMP)/sanitation standard operating procedure (SSOP), must be established before the implementation of the HACCP (hazard analysis and critical control point) system that more closely controls the risks to human health, as well as the prevention of modifications of foodstuffs by means of control practices in all stages of white meat production (de Oliveira et al., 2016). GHP/GMP/SSOP programs are based on the exclusion and elimination of unwanted and foreign materials, with the inhibition and destruction of pathogenic microorganisms. The integration of GHP/GMP/SSOP programs with the HACCP system ensures process hygiene and impacts on meat safety, thereby helping control foodborne diseases (de Oliveira et al., 2016).

5. Conclusion

In conclusion, according to the results of this study, the highest score (4) was the most common in cases of pododermatitis. Our results revealed the involvement of six bacterial species in pododermatitis in broiler chickens: *S. aureus*, *S. hyicus*, *E. coli*, *S. flexneri*, *S. sonnei,* and *S. enterica*. Staphylococci have been identified as being more common than enterobacteria in pododermatitis lesions, with *S. aureus* being the primary causative agent. A single pododermatitis lesion can be co-infected with up to five bacterial species, although the majority of pododermatitis lesions are infected with a single bacterial species.

The results of this study demonstrate that cleaning/washing feet with acetic acid significantly decreased the prevalence of pathogenic bacteria. It is imperative to establish GMP and SSOP programs, followed by the HACCP system, to help reduce the presence of pathogenic bacteria in the poultry slaughterhouse environment. In turn, the chicken meat produced will carry lower levels of contamination with pathogenic microorganisms. It should therefore be safer from a public health point of view, improving animal welfare.

Conflict of Interest: The authors declare no potential conflicts of interest.

6. References

Abdul Wahid AT. Effects of acetic acid and hydrogen peroxide on the microbiological quality and skin appearance of poultry carcasses. Basrah Journal of Veterinary Research, 7:(2);97-100, 2008.

Amrutha B, Sundar K, Shetty PH. Effect of organic acids on biofilm formation and quorum signaling of pathogens from fresh fruits and vegetables. Microbial Pathogenesis, 111;156-162, 2017.

Chaudhary S, Batoo AS, Hussain K, et al. Prevalence of pododermatitis caused by *Staphylococcus aureus* in poultry birds of Jammu. International Journal of

Livestock Research,8:(3);192-195, 2018. https://doi.org/10.5455/ijlr.20170818064853

CLSI (Clinical and Laboratory Standards Institute). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. M7-A9. 9thed. Wayne, PA. 2018.

De Jong IC, van Harn J, Gunnink H, et al. Footpad dermatitis in Dutch broiler flocks: Prevalence and factors of influence. Poultry Science, 91;1569-1574. 2012. http://dx.doi.org/10.3382/ps.2012-02156

De Oliveira CAF, da Cruz AG, Tavolaro P, et al. Food Safety: Good Manufacturing Practices (GMP), Sanitation Standard Operating Procedures (SSOP), Hazard Analysis and Critical Control Point (HACCP). In Antimicrobial Food Packaging. Academic Press. pp. 129-139. 2016. https://doi.org/10.1016/B978-0-12-800723-5.00010-3

Derbal S, Hanfer M. Biofilm production and acetic acid sensitivity of Staphylococcus aureus and Escherichia coli isolated from poultry slaughterhouse environment, broiler carcasses and offal in Algeria. Meat Technology, 65:(2);113-129. 2024.https://doi.org/10.18485/meattech.2024.65.2.5

Dinev I, Denev S, Vashin I, et al. Pathomorphological investigations on the prevalence of contact dermatitis lesions in broiler chickens. Journal of Applied Animal Research, 47:(1);129-134. 2019. https://doi.org/10.1080/09712119.2019.1584105

Ducková V, Kročko M, Tkáčová J. Microbial biofilms in a meat processing environment. Meat Technology, 64:(2);508-512. 2023. https://doi.org/10.18485/meattech.2023.64.2.98

Fraise AP, Wilkinson MAC, Bradley CR, et al. The antibacterial activity and stability of acetic acid. Journal of Hospital Infection, 84:(4);329-331. 2013. https://doi.org/10.1016/j.jhin.2013.05.001

Hashimoto S, Yamazaki K, Obi T, et al. Footpad dermatitis in broiler chickens in Japan. Journal of Veterinary Medical Science,73:(3);293-297. 2011. https://doi.org/10.1292/jvms.10-0329

Hashimoto S, Yamazaki K, Obi T, et al. (2013). Relationship between severity of footpad dermatitis and carcass performance in broiler chickens. Journal of Veterinary Medical Science,75:(11);1547-1549. https://doi.org/10.1292/jvms.13-0031

Jacob F, Baracho M, Nääs I, et al. Risk of incidence of hock burn and pododermatitis in broilers reared under commercial conditions. RevistaBrasileira de Ciência do Solo,18(3):357-362. 2016. https://doi.org/10.1590/1806-9061-2015-0183

Markey BK, Leonard FC, Archambault M, et al. Clinical Veterinary Microbiology, 2nd edition, Mosby Elsevier. 2013.

Martins B, Martins M, Mendes A, et al. Footpad dermatitis in broilers: differences between strains and gender. RevistaBrasileira de Ciência do Solo,18(3):461-466. 2016. https://doi.org/10.1590/1806-9061-2015-0105

Martrenchar A, Boilletot E, Huonnic D, et al. Risk factors for foot-pad dermatitis in chicken and turkey broilers in France. Preventive Veterinary Medicine,52:(3-4);213-226. 2002. https://doi.org/10.1016/s0167-5877(01)00259-8

Mayne RK. A review of the aetiology and possible causative factors of foot pad dermatitis in growing turkeys and broilers. Worlds Poultry Science Journal,61:(2);256-267. 2005. https://doi.org/10.1079/WPS200458

Olsen RH, Christensen H, Kabell S, et al. Characterization of prevalent bacterial pathogens associated with pododermatitis in table egg layers. Avian Pathology,47:(3);281-285. 2018. https://doi.org/10.1080/03079457.2018.1440066

Ouattara B, Simard RE, Holley RA, et al. Inhibitory effect of organic acids upon meat spoilage bacteria. Journal of Food Protection, 60:(3);246-253. 1997. https://doi.org/10.4315/0362-028X-60.3.246

Piller A, Bergmann S, Schwarzer A, et al. Validation of histological and visual scoring systems for foot-pad dermatitis in broiler chickens. Animal Welfare,29:(2);185-196. 2020. https://doi.org/10.7120/09627286.29.2.185

Szafraniec GM, Szeleszczuk P, Dolka B. Review on skeletal disorders caused by Staphylococcus spp. in poultry. Veterinary Quarterly,42:(1);21-40. 2022. https://doi.org/10.1080/01652176.2022.2033880

Tamblyn KC, Conner DE. Bactericidal activity of organic acids against Salmonella typhimurium attached to broiler chicken skin. Journal of Food Protection, 60:(6);629-633. 1997. https://doi.org/10.4315/0362-028X-60.6.629

Taufik M, Triatmojo S, Erwanto Y, et al. Effect of broiler age and extraction temperature on characteristic chicken feet skin gelatin. The 5th International Seminar on Tropical Animal Production. Community Empowerment and Tropical Animal Industry, October 19-22, Yogyakarta, Indonesia. 2010.

Thøfner ICN, Poulsen LL, Bisgaard M, et al. Correlation between footpad lesions and systemic bacterial infections in broiler breeders. Veterinary Research,50:(1);1-5. 2019. https://doi.org/10.1186/s13567-019-0657-8

Tiona NA, Rahelivololoniaina BR. Broiler Feet Gelatine. Engineering, 15;234-241. 2023. https://doi.org/10.4236/eng.2023.154018

Welfare Quality. Welfare Quality Assessment Protocol for Poultry (Broilers, Laying Hens). Welfare Quality Consortium, Lelystad, the Netherlands. 2009.

Youssef FM, Soliman AA, Ibrahim GA, et al. Advanced bacteriological studies on bumblefoot infections in broiler chicken with some clinicopathological alteration. Veterinary Sciences Research,1:(1);1-9. 2019.

Zhao T, Doyle MP. Reduction of Campylobacter jejuni on chicken wings by chemical treatments. Journal of Food Protection, 69:(4);762-767. 2006. https://doi.org/10.4315/0362-028x-69.4.762

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On-farm Phenotypic Characterization of Indigenous Goat Types in Selected Districts of South Gondar Zone, North Western Ethiopia

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Abstract: This study aimed to gather basic information on phenotypic characteristics and develop live weight estimation equations based on body measurements of indigenous goat types in selected districts of the South Gondar Zone. Multistage purposive sampling was employed based on the potential of goat production. For generating quantitative and qualitative data, linear body measurements were taken from 630 mature goats. Dentition was used to estimate the age of goats as age group one (1PPI), age group two (2PPI), and age group three (3PPI). Data were gathered through field observations and linear body measurements of sampled populations. The most frequently observed coat colors were white (23.80%), white and red (18.57%), red (13.70%), gray (13.17%), brown (9.50%), and black (6.03%). Moreover, the most frequently observed coat color patterns of the sampled goat population in the study area were plain (65.1%), followed by patchy (25.2%) and spotted (9.7%). Sex of animals affected ($P<0.05$) body weight, and all of the body measurements except for ear length and tail length. The animals' location difference affected all body measurements ($P < 0.05$), except for heart girth. The age of animals contributed significantly to differences in body weight and all linear body measurements. The correlation coefficient was consistently highest between body weight and chest girth in males ($r = 0.90$) and females ($r = 0.83$). Multiple regression analysis revealed that Chest girth was the most crucial trait in predicting body weight. There was no standard weighing balance for selling live animals at reasonable prices.
Keywords: Linear Body Measurements; Multiple linear regression analysis; Quantitative and qualitative traits.

1. Introduction

In Ethiopia, livestock is an essential and integral component of agriculture, the backbone of the economy in terms of its contributions to agricultural value-added and national GDP (ILRI, 2011). Ethiopia is one of Africa's largest countries in terms of livestock populations, comprising 54 million goats, 71 million cattle, 43 million sheep, 13.33 million equines, 7 million bee colonies, and 57 million chickens, excluding nomadic areas, and is genetically diverse. Although Ethiopia has a diverse range of large and small ruminants, their productivity has been constrained by numerous complex challenges. Indigenous goat breeds constitute over 95% and 99.77% of the small ruminant population of Africa and Ethiopia, respectively (CSA, 2022). In Ethiopia, the distribution of goats varies between highland and lowland areas. Approximately half of the goat population is kept by pastoralists in the lowlands, while the remaining half is found in small flocks on mixed highland farms. This distribution highlights the significant role goats play in pastoral and mixed farming systems across the country. Goats have a short reproductive cycle and a high multiplication rate compared to large ruminants.

Having developed certain valuable genetic traits, such as the ability to perform better under low conditions and climatic stress, tolerance to infectious diseases and parasites, as well as heat stress, these peculiar traits enable goats to cope with the stressful nature of the vast marginal lands in the region. Goats also have socio-cultural roles in traditional societies. They have often been referred to as the 'poor man's cow' and serve as an essential source of savings for the agrarian community, particularly in developing countries (Seyed et al., 2014). Understanding the existing diversity of management strategies among small-scale keepers (housing, watering, feeding, and health control) and their associated challenges would help develop effective intervention strategies. Characterizing a local genetic resource based on morphological traits plays a crucial role in the classification of animals, particularly in terms of size and shape, which can serve as a reasonable economic indicator (Okpeku et al., 2011). The goat population of Ethiopia has been phenotypically classified into 11 distinct kinds or major breed types, as well as five additional subtypes. However, genetic and molecular characterization revealed only eight distinct breed types or populations in the country. It's well understood that morphological measurements are a critical method used to evaluate and assess the characteristics of various animal breeds. These measurements can help to provide basic information on the suitability of the animals for their selection (Yakubu, 2010). Although phenotypic characterization is vital for breed identification and classification, it is relatively shallow in the South Gondar Zone. Moreover, previous works done by some scholars (Birara et al., 2021; Belete et al., 2022) are not detailed enough to fully explain the potential and physical characteristics of the breed in the zone.

Furthermore, it was area-specific in its coverage and expression of the potential of these animals. In addition to these facts, breed characterization is a prerequisite for animal conservation, documentation, and proper utilization (FAO, 2012; Gizaw et al., 2011). Moreover, the study area has a diversified goat population, but phenotypic characterization has not been conducted so far,

particularly for indigenous goat breed types. Thus, this study aimed to phenotypically characterize indigenous goat types in some selected districts of the South Gondar Zone.

2. Materials and Methods

2.1. Description of the study area

The study was conducted in three districts of the South Gondar Zone, Amhara National Regional State, Ethiopia.

2.1.1. Tach Gayint

It is located in the northeast of Bahir Dar town at a distance of 200 km. The district is bounded by the Lay Gayint district to the north, Simada to the west, the Checheho River to the east, and the Bashilo River to the south (SGADO, 2024). According to SGADO (2024), the district is characterized agro-ecologically as moist *Woina Dega,* with an annual rainfall range of 900 to 1000 mm and a temperature range of 13 to 27 °C. Topographically, the flat area accounts for 24%, the mountainous and hilly areas for 63%, and the valley bottom area for 13%. It lies between 11°21′ and 11°05′ north latitude and 28° 20′ and 28° 42′ east longitude, and at an altitude of 750-2800 meters above sea level (masl). The livestock population in the district is estimated to be 28,429 cattle, 73,658 sheep, 81,962 goats, 18,285 equines, 1,305 beehives, and 123,452 poultry (SGADO, 2024).

2.1.2. Simada

It is located at 11°30′ N latitude and 38°15′ E longitude. The district is bordered by the Bashilo River in the southeast, the Abay River in the southwest, the East Estie district in the west, the Lay Gayint in the north, and the Tach Gayint in the northeast. The district is classified as one of the food-insecure areas within the zone due to recurrent climatic and agricultural challenges (MoA, 2023). The total area of the district is approximately 2,244.96 square kilometers, comprising 10% highland, 30% midland, and 60% lowland areas. Elevation ranges from 750 to 3,500 meters above sea level, with an annual temperature range of 15 to 25 °C and an average annual rainfall between 700 and 1,000 mm (NMA, 2023). The soil composition consists of 55% brown, 20% reddish, and 25% black soils, supporting a diverse range of agricultural activities (Abebe et al., 2024). The predominant land use pattern indicates that 145,251 hectares are allocated for cultivation, 12,680 hectares are covered by forest, and 48,291 hectares are used for grazing (SGADO, 2024). The main crops grown include teff, millet, maize, wheat, barley, potatoes, beans, chickpeas, linseed, sorghum, apples, garlic, onions, and haricot beans. Livestock resources are substantial, with populations comprising 58,647 cattle, 92,841 sheep, 96,521 goats, 23,524 equines, 192,658 poultry, and 961 beehives (SGADO, 2024; CSA, 2024).

2.1.3. East Estie

It is one of the districts in the Amhara National Regional State of Ethiopia. Part of the south Gondar zone. It is bordered on the south by the Abay River, on the west by the Estie and Dera districts, on the northwest by the Fogera district, on the east by the Simada district, on the northeast by Lay Gayint, and on the north by the Farta district. The administrative center is Mekane Eyesuse (SGADO, 2024).

2.2. Sampling techniques

A multistage purposive sampling technique was employed to capture the diversity and potential of goat production across the South Gondar Zone. Initially, 16 districts within the zone were assessed based on their ecological suitability, existing goat population, and the relative importance of goat production in local livelihoods. From these three districts, Tach Gayint, Simada, and East Estie, were purposively selected due to their high goat production potential and representation of varied agro-ecological zones. Following district selection, a total of 630 indigenous goats were randomly sampled from selected households to obtain data on both linear body measurements and qualitative physical characteristics. This sampling approach ensured representative coverage of phenotypic traits across the study areas (Tsegaye et al., 2024).

2.3. Methods of data collection

The goats were classified into three age groups based on their dentition, using the following criteria: 1 PPI (one pair of permanent incisors), 2 PPI (two pairs of permanent incisors), and 3 PPI (three pairs of permanent incisors) to represent ages of less than 2 years, 2–3 years, and more than 3 years, respectively (Wilson and Durkin, 1984; Tesfaye et al., 2021). Moreover, morphological characters (qualitative) and body measurements (quantitative) were collected, and data were recorded in the prepared format. Morphological characters, including coat color pattern, coat color type (CCT), head profile (HP), ears, wattles, horns, ruff, and tail, were observed and recorded using standard color charts (FAO, 2012). Furthermore, quantitative traits, including chest girth (CG), body length (BL), wither height (WH), ear length (EL), horn length (HL), rump length (RL), scrotal circumference (SC), and tail length (TL), were measured using a plastic measuring tape. Body weight (BW) was recorded using a spring balance with a 50 kg capacity and 0.2 kg precision (Yimer et al., 2023; Hagos et al., 2022).

2.4. Data management and analysis

Morphological and quantitative Data collected from each site were carefully coded and entered into a Microsoft Excel sheet for further analysis. Preliminary data analysis, including homogeneity testing, normality assessment, and outlier screening, was conducted before the primary data analysis for male and female goats within breed and location using the frequency procedure in the Statistical Analysis System (SAS, version 9.1.3). Quantitative character (body weight and linear body measurements) was analyzed using the GLM procedures of SAS Software.

The model used to analyze adult body weight and other linear body measurements was:

$$Y_{ijk} = \mu + D_i + S_j + A_k + (SA)_{jk} + e_{ijk}$$

Y_{ijk} = the observed body weight and linear body measurements
μ = overall mean
D_i = is the effect of the i^{th} district (Tach Gayint, Simada, and East Estie)
S_j = is the effect j^{th} of the jth sex (female and male)
A_K = is the effect k^{th} of age group (1PPI, 2PPI, and 3PPI)
$(SA)_{jk}$ = is the interaction effect jk^{th} of sex and age
e_{ijk} = is the random residual error.

Moreover, Pearson's correlation coefficients were used to measure the strength of the relationship between BW and other linear body measurements (LBM) within the same sex and age group. Body weight and other LBM: CG, BL, WH, EL, HL, TL, RL, and SC were included for males but excluded for female goats. Furthermore, the regression procedures in SAS (2008) were used to determine the best-fit regression equation for predicting BW from other LBMs. Thus, the following multiple linear regression models were used to regress BW from different LBM.

For males:

$$Y_j = \alpha + \beta_1X_1+\beta_2X_2+ \beta_3X_3+ \beta_4X_4 +\beta_5X_5+\beta_6X_6+\beta_7X_7+ \beta_8X_8 + e_j$$

Where:

Y_j = the response variable; body weight
α = the intercept
X_1, X_2, X_3, X_4, X_5, X_6, X_7, and X_8 are the explanatory variables BW, WH, CG, TL, SC, EL, RL, and HL.
B_1, β_2, …, β_8 is the regression coefficient of the variables X_1, X_2,..., X_8
e_j = the residual random error

For females:

$$Y_j = \alpha + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_5X_5 + \beta_6X_6 +\beta_7X_7 + e_j$$

Where:

Y_j = the dependent variable, body weight
α= the intercept
X_1, X_2, X_3, X_4, X_5, X_6, and X_7 are the independent variables: BW, WH, CG, TL, EL, RL, and Hl.
β_1, β_2, ..., β_7 is the regression coefficient of the variable X_1, X_2,...,X_7
e_j = the residual random error.

3. Results and Discussion

3.1. Qualitative physical characteristics

The coat color pattern among the studied indigenous goat population was predominantly plain (65.1%), followed by patchy (25.23%) and spotted (9.7%) (Table 1). In contrast, Halima et al. (2012) documented a higher frequency of spotted coat patterns (36.1%) in six Ethiopian goat populations, followed by patchy (32.4%) and plain (30.4%) patterns, indicating regional variation in phenotypic traits. In the present study, the most common primary coat color was white (23.8%), followed by white and red (18.57%), red (13.7%), and gray (13.17%). However, a higher prevalence of white coat color (42.5%) was reported in goats from the Babile district in the Oromia region (Mahilet, 2012). Regarding hair type, the dominant coat was short and smooth (79.2%), with a smaller proportion of goats exhibiting long and coarse hair (18.25%). Only 2.54% of the population had a short and coarse hair type. Most goats had straight horns (55.6%), while curved horns accounted for 36.5%. Horn orientation was predominantly backward (59.7%), with the remaining 40.3% showing oblique upward orientation. Ear orientation was pendulous primarily (63.0%), followed by semi-pendulous (28.3%) and erect (8.7%). The head profile distribution showed a high prevalence of concave profiles (73.7%), with smaller proportions exhibiting slightly concave (16.2%) and straight (10.2%) profiles. These variations in morphological traits may stem from unregulated mating practices and gene flow between populations resulting from market-mediated animal movement. Comparable results were reported by Guang-Xin et al. (2018) in Chinese goats, where human-mediated exchanges drove gene admixture. Al-Araimi et al. (2019) also found evidence of population admixture in Omani goats from neighboring regions, underscoring the influence of proximity and movement on genetic and phenotypic diversity. Recent studies by Getachew et al. (2022) and Tadesse et al. (2023) have further confirmed the influence of local ecological and socio-economic factors on the phenotypic variability of indigenous goats in Ethiopia, emphasizing the need for site-specific characterization to facilitate effective conservation and breeding interventions.

Character	Tach Gayint		Simada		East Estie		Overall	
	N	%	N	%	N	%	N	%
Body hair coat color pattern								
Plain	140	72.2	138	62.72	132	61.68	410	65.1
patchy (pied)	44	22.7	60	27.27	55	24.77	159	25.23
Spotted	10	5.2	22	10	29	13.55	61	9.7
X^2 value								50.8*
Body hair coat color type								
white	68	35.1	37	16.8	45	21.03	150	23.80
red	34	17.53	41	18.6	11	5.14	86	13.7
black	6	3.1	4	1.82	28	13.08	38	6.03
brown	15	7.7	40	18.18	5	2.34	60	9.5
gray	20	10.3	19	8.64	44	20.56	83	13.17
white and red	30	15.5	38	17.27	49	22.9	117	18.57
White and black					5	1.4	5	1.3
White and brown			7	3.18			7	1.11
White and gray	10	5.2	16	7.27	6	28	32	5.1
All color dominant	11	5.7	18	8.18	23	10.65	52	8.3
X^2 value								101.63*
Hair coat type								
short and smooth	135	69.6	163	74.1	201	93.1	499	79.20
long and course	45	23.2	57	25.91	13	6.01	115	18.25
short and course	14	7.2			2	0.9	16	2.54
X^2 value								63.03*
Horn shape								
Straight	89	45.88	141	64.1	120	55.56	350	55.6
Curved	76	39.18	62	28.18	92	45.59	230	36.5
X^2 value								31.54*
Horn orientation								
obliquely upward	82	42.27	91	41.4	81	37.5	254	40.3
backward	112	57.73	129	58.6	135	62.5	376	59.7
X^2 value								0.97ns
Ear orientation								
Erect	5	2.58	14	6.36	36	16.67	55	8.7
Pendulous	148	76.29	167	75.91	82	37.96	397	63.0
semi-pendulous	41	21.13	39	17.73	98	45.37	178	28.3
X^2 value								139.75*
Facial (head) profile								
Straight	21	10.82	25	11.36	18	8.33	64	10.2
Concave	156	80.41	141	64.10	167	77.31	464	73.7
Slightly concave	17	8.76	54	24.55	31	14.35	102	16.2
X^2 value								23.84*
Wattles								
Absent	180	92.78	212	96.36	209	96.76	601	95.40
Present	14	7.22	8	3.64	7	3.24	29	4.60
X^2 value								2.79^{ns}
Ruff								
Absent	172	88.66	183	83.18	172	79.63	527	83.7
Present	22	11.34	37	16.82	44	20.37	103	16.3
X^2 values								6.04*

Table 1 – Qualitative traits of goat population in the study areas; *= significant, ns = non-significant.

3.2. Multiple Correspondence Analyses

Multiple correspondence analyses were carried out for nine (9) qualitative traits recorded to understand the typical features of each district's indigenous goat types morphologically. Figure 1 shows a bi-dimensional graph representing the associations among the categories of the analyzed qualitative traits. The association is based on points located in approximately the same direction from the origin, in a roughly similar region of space. From the figure, it can be seen that 28.28% of the total variation was explained by the first two dimensions (16.98% by the first and 11.30% by the second dimension). On the identified dimensions, the sample goat

population in East Estie district clustered together with plain coat color pattern with white, red, and white red coat color type, medium hair length, presence of horn and straight horn shape, semi pendulous and pendulous ear orientation backward and obliquely horn orientation, absence of wattles, flat and sloping rump profile, and straight back profile. The goat population in the Simada district was also closely associated with plain coat color patterns, including white, black, and fawn coat colors, smooth hair coat types, semi-pendulous and pendulous ear orientations, and concave and straight facial profiles. The multiple correspondence analysis revealed that the goat population found in the Estie, Simada, and Tach Gayint districts shares some similar morphological characteristics along the identified dimensions. This might be due to the geographic proximity of the three districts.

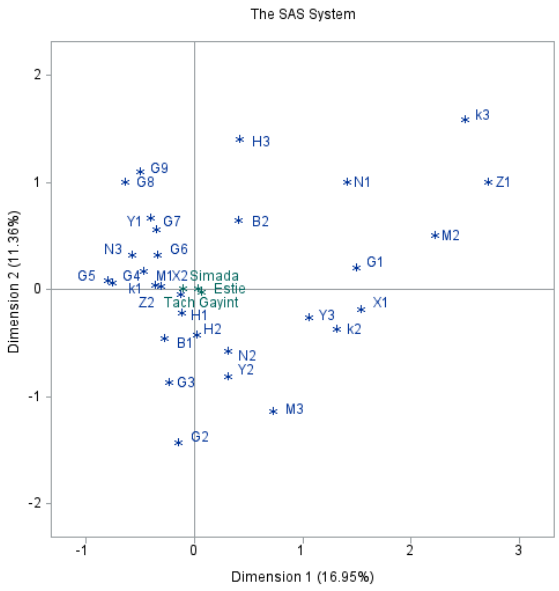

Figure 1 – A bi-dimensional plot shows the associations among different categories of qualitative variables.

3.3. Quantitative physical characteristics

Table 2 shows the BW and LBMs for female and male goats at various ages in different locations.

3.3.1. Sex effect

In all three study districts, sex had a statistically significant effect ($P < 0.05$) on most of the measured linear body traits, including BW, BL, CG, HW, HL, and RL. However, EL and TL did not show significant differences between sexes. Across all traits with substantial differences, male goats consistently exhibited higher mean values than their female counterparts. The observed higher values of quantitative traits in male goats can primarily be attributed to physiological and endocrinological differences between the sexes. Notably, the influence of anabolic hormones, such as testosterone, in males enhances muscle growth and skeletal development, contributing to superior growth performance and a larger body conformation compared to females (Gichohi-Wainaina et al., 2023). Moreover, males are less burdened by reproductive physiological demands, which may allow more resources to be directed toward somatic growth (Tesfaye et al., 2021). These findings align with several other studies that have reported sex-related differences in phenotypic traits of indigenous goat populations across diverse agro-ecological zones in Ethiopia and other parts of sub-Saharan Africa (Duguma et al., 2022; Hassen et al., 2020). Understanding such differences is important for designing targeted breeding programs and improving productivity in indigenous goat populations.

3.3.2. Age effect

In the sample goat population, age was strongly influenced ($p<0.01$) by all measurements except TL and EL. Body weight, BL, CG, and WH in all age groups were significantly higher ($p<0.01$) in age group 3PPI than in 1PPI and 2PPI. There was wide variability in these body measurements as the age of the animals increased. This implies that these variables best explain the growth pattern of the animals. On the contrary, variables such as ear length and tail length were less influenced by age and showed less variation as age advanced. Similarly, Belete et al. (2022) also reported higher BW, BL, CG, and WH than all age groups in the Lay Gaint and Simada districts of South Gonder zone goat populations. The higher LBMs observed at the maturity stage may be due to muscle development. Moreover, Birara et al. (2021) also reported higher LBMs in both sexes for goat populations in the Farta, Fogera, and Libokemkem districts of the South Gondar zone.

3.3.3. Location effect

Location had a statistically significant influence on BW, BL, HW, HL, SC, and RL, while CG, EL, and TL remained unaffected. The observed spatial differences in linear body measurements (LBMs) could be attributed to disparities in the availability and quality of feed resources across the study sites. In addition, household-level differences in animal husbandry practices, such as housing, healthcare, and feeding regimes, may have contributed to the variability in these traits among the sampled sheep populations. These

findings align with recent studies emphasizing the role of environmental and management conditions in shaping phenotypic variation in small ruminants (Gebremedhin et al., 2023; Tsegaye et al., 2022).

3.3.4. Sex-by-age interaction effect

The interaction of sex and age group was significant ($p<0.01$) for CG, BL, BW, HW, and RL, except for EL and TL. In each age group, males had higher values in BW, BL, HW, and HL. The variation in these LBMs between sex and age may be due to variations in measurements at an advanced stage of the animals.

	CG	WH	BL	BW	EL	TL	HL	SC	RL
	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE
Overall	63.73±0.22	60.09±0.02	55.22±0.21	29.34±0.28	20.36±0.09	12.12±0.07	16.82±0.07	5.6±0.8	12.23±0.06
R^2	0.48	0.36	0.41	0.49	0.05	0.08	0.29	0.54	0.44
CV%	4.73	5.39	5.79	13.06	9.13	12.32	6.86	20.83	9.19
Age group	*	*	*	*	NS	NS	*	*	*
1 PPI	58.05±0.46^{c}	55.59±0.49^{c}	49.64±0.48^{c}	22.18±0.58^{c}	19.48±0.28^{c}	11.96±0.22^{b}	15.2±0.17^{c}	NA	12.30±0.09^{bc}
2PPI	63.22±0.45^{b}	59.49±0.48^{b}	55.08±0.47^{b}	28.87±0.57^{b}	20.27±0.28^{b}	11.89±0.22^{bc}	16.97±0.17^{b}	NA	12.62±0.09^{b}
3PPI	67.38±0.39^{a}	63.45±0.41^{a}	58.33±0.41^{a}	34.72±0.49^{a}	21.14±0.23^{a}	12.64±0.19^{a}	17.76±0.15^{a}	NA	13.58±0.15^{a}
Age by Sex	**	**	**	**	NS	NS	*	Ns	*
1PPI female	56.59±0.42^{d}	53.98±0.45^{d}	47.94±0.45^{f}	20.23±0.53^{e}	19.33±0.26^{c}	10.98±0.21^{c}	14.83±0.16^{e}	NA	11.77±0.1^{c}
2PPI female	60.81±0.36^{c}	58.2±0.39^{c}	52.92±0.39^{d}	25.81±0.47^{d}	19.94±0.22^{bc}	12.07±0.18^{b}	16.19±0.14^{c}	NA	12.56±0.1^{bc}
3PPI female	64.07±0.30^{b}	60.49±0.32^{b}	55.64±0.32^{c}	29.77±0.38^{c}	20.56±0.18^{b}	12.04±0.15^{b}	16.72±0.11^{b}	NA	12.92±0.11^{b}
1PPI male	59.51±0.79^{c}	57.19±0.85^{c}	51.33±0.84^{e}	24.13±1.01^{d}	19.63±0.48^{c}	12.94±0.39^{a}	15.56±0.3^{d}	27.5±7.8	12.83±0.15^{b}
2PPI male	65.63±0.80^{b}	60.77±0.86^{b}	57.22±0.85^{b}	31.93±1.03^{b}	20.61±0.49^{b}	11.71±0.4^{b}	17.74±0.3^{a}	28.7±5.9	12.68±0.16^{bc}
3PPI male	70.70±0.70^{a}	66.43±0.75^{a}	61.03±0.74^{a}	39.67±0.89^{a}	21.73±0.43^{a}	13.25±0.34^{a}	18.8±0.27^{a}	26.9±6.6	14.24±0.28^{a}
Location	Ns	*	*	*	NS	NS	*	*	*
Tach Gayint	64.5±0.72^{a}	60.86±0.24^{a}	54.99±0.24^{b}	36.73±0.46^{a}	20.39±0.14	12.18±0.11	17.3±0.09^{a}	24.7±0.7^{b}	13.19±0.09^{a}
Simada	65.01±0.36^{a}	61.44±0.39^{a}	55.91±0.38^{a}	36.29±0.29^{a}	20.46±0.22	12.44±0.17	16.8±0.14^{b}	25.2±0.5^{b}	12.36±0.08^{b}
East Estie	63.93±0.79^{a}	60.15±0.31^{b}	54.95±0.31^{b}	31.27±0.37^{b}	19.42±0.18	11.31±0.14	16.05±0.11^{c}	26.9±1^{a}	11.60±0.9^{c}
Sex	*	*	*	*	Ns	Ns	*	*	*
Male	67.81±0.41^{a}	63.59±0.45^{a}	58.42±0.44^{a}	35.45±0.53^{a}	20.73±0.25^{a}	12.69±0.2^{a}	17.9±0.16^{a}	28.07±0.67	12.06±0.06^{b}
Female	62.06±0.2^{b}	58.71±0.21^{b}	53.47±0.21^{b}	27.09±0.25^{b}	20.13±0.12^{a}	11.93±0.09^{a}	16.2±0.07^{b}	NA	12.71±0.1^{a}

Table 2 – Least square mean (±SE) for body weight and linear body measurements; $^{a,b,\ c,d,e,f,}$ means on the same column with different superscripts within the specified class variable, are significantly different ($p<0.05$) NA = not applicable *=significant $P< 0.05$; **= significant ($p<0.01$), ns= non-significant, BW= body weight, CG=heart Girth, BL= body length, HW= Height at wither, TL=tail length, HL= Horn length, EL=ear length, SC =scrotum circumference, RL = Rump length, SE=standard error.

3.4. Materials e Methods

Pearson's correlation coefficients among the quantitative body measurements of indigenous goats, categorized by sex, are presented in Table 3. The results demonstrated that body weight (BW) was significantly and positively correlated with almost all continuous traits assessed in both male and female goats, except for tail length (TL), which showed a weak and inconsistent association. In male goats, BW exhibited strong correlations with heart girth (HG), body length (BL), height at withers (HW), head length (HL), and rump length (RL), with coefficients of 0.90, 0.86, 0.84, 0.69, and 0.55, respectively. Similarly, in females, BW was also strongly correlated with HG (0.83), BL (0.79), HW (0.77), HL (0.50), and RL (0.46). Among these, heart girth consistently showed the highest correlation with BW in both sexes, followed by BL and HW. This strong association between BW and HG suggests that HG can serve as a reliable proxy trait for estimating live body weight in indigenous goats, especially under field conditions where weighing scales are not readily available.

The high correlation between BW and other linear body measurements (LBMs) such as BL, HW, and HL further reinforces the value of morphometric traits in evaluating the growth and productivity of indigenous goats. These findings align with recent studies that emphasized the predictive power of morphometric measurements in estimating BW in local goat populations across various agro-ecological zones. For instance, Tesfaye et al. (2023) reported similar high correlations between BW and HG, BL, and HW in Ethiopian indigenous goats. Likewise, Alade and Onakpa (2022) demonstrated the utility of HG and BL as effective predictors of BW in West African Dwarf goats. A more recent study by Abegaz et al. (2024) validated these associations in highland goat populations, emphasizing the practical application of LBMs in breed characterization and management interventions.

Overall, the results of this study highlight the importance of simple, non-invasive measurements such as HG, BL, and HW in predicting live BW. This is especially useful for smallholder farmers and extension agents in rural areas, enabling better decision-making in selection, nutrition, and health management practices without the need for expensive equipment.

	BW	HG	BL	HW	HL	TL	EL	RL	SC
BW		0.83*	0.79*	0.77*	0.50*	0.24^{NS}	0.28*	0.46*	NA
HG	0.90*		0.69*	0.74*	0.49*	0.22^{NS}	0.22*	0.44*	NA

BL	0.86*	0.82*		0.75*	0.49*	0.21^{NS}	0.24*	0.51*	NA
HW	0.84*	0.85*	0.84*		0.45*	0.22^{NS}	0.30*	0.31*	NA
HL	0.69*	0.70*	0.72*	0.42*		0.25^{NS}	0.25*	0.52*	NA
TL	0.17**	0.21**	0.25*	0.16**	0.32*		0.12*	0.19*	NA
EL	0.32*	0.33*	0.26*	0.26*	0.28*	0.21^{NS}		0.19*	NA
RL	0.55*	0.59*	0.46*	0.35*	0.56*	0.40^{NS}	0.26*		NA
SC	-0.25**	0.12*	0.126*	0.12*	0.01*	0.33^{NS}	-0.02*	0.35*	

Table 3 – The correlation coefficient among body weight and linear body measurements (values above the diagonal were for females while below the diagonal were for males): LBM= linear body measurements, HG =heart girth, BL = body length, height at wither, HL = head length, BW =body weight TL= tail length, EL= ear length, SC= scrotum circumference, RL = rump length, **= significant (p<0.01), *=significant (p<0.05). NA = not applicable, NS = non-significant.

3.5. Prediction of live body weight from linear body measurements

Multiple regression equations were developed for male (Table 4) and female (Table 5) goat populations to predict BW from LBMs. The LBMs used in the present study were HG, BL, HW, HL, TL, RL, and EL. For optimum model selection, prescreening is advisable to remove the least contributing model terms. A multiple regression procedure was carried out within each sex group on independent variables positively correlated with body weight to predict BW from LBMs. The regression equation was developed to estimate body weight using seven linear body measurements (HG, BL, HW, HL, TL, RL, and EL) in female and male goats. Variables that best fitted the model were selected using the C(p) statistic, R^2 (adjusted R-square), MSE (mean square of error), Schwarz Bayesian criteria (SBC), and Akaike's information criterion (AIC). One may define the "best model" as that which has a small value of C(p), which is also close to p (the number of parameters in the model, including the intercept), with the "highest adjusted value, "the highest Schwarz Bayesian Criteria (SBC) and the highest Akaike's information criteria (AIC). The small Cp indicates precision and small variance in estimating the population regression coefficients, while the coefficient of determination (R^2) represents the proportion of the total variability the model explains. Chest girth was consistently selected as the best-fitting variable among the others. Thus, CG, BL, HW, and EL were chosen for the female goat, while for male goats, CG, BL, and TL were the best-fitted variables for predicting BW. The prediction of BW could be based on a regression equation developed for both males and females, using CG and other variables (CG, BL, and TL for males) and (CG, BL, HW, and EL for females). According to Hlokoe et al. (2022), regression analysis is a crucial tool in livestock research for determining the relationship between the quantitative response variable and explanatory variables, such as BW and LBM.

This mechanism of analysis is more critical in the absence of weighing balances. Thus, the best-predicted equation where there is no weighing balance at farmers' management condition for the estimation of BW from LBMS was BW= -24.88+0.45HG+0.16BL+0.05HW+0.30EL and BW= -11.57+0.35HG+0.19BL+0.13TL for females (Table 4) and males (Table 5) indigenous goat population in south Gonder Zone, respectively. A similar conclusion was drawn in the study by Hlokoe et al. (2022), which indicated that body weight can be accurately predicted by considering multiple biometric traits. Based on this, it is recommended that traits such as heart girth, withers height, sternum height, rump height, head length, ear length, rump width, and body length be incorporated into breeding programs to improve body weight in male Nguni cattle. For female Nguni cattle in India, heart girth, rump height, and wither height should be prioritized as selection criteria for predicting body weight. Moreover, a previous study by Odadi (2018) found that heart girth is the most reliable indicator for estimating the body weight of heifers in Northern Kenya. Similarly, Ashwini et al. (2019) determined that heart girth, withers height, hip height, and head width are crucial factors in determining the body weight of crossbred cattle in India.

	I	β1	β2	β3	β4	R^2	A-R^2	CP	AIC	RMSE	SBC
CG	-22.82	0.66				0.75	0.75	93.42	654.20	1.93	661.82
CG+BL	-25.17	0.51	0.20			0.77	0.77	5.80	574.62	1.81	609.32
CG+BL+HW	-23.93	0.46	0.16	0.32		0.78	0.78	43.03	610.39	1.76	588.50
CG+BL+HW+EL	-24.88	0.45	0.16	0.05	0.30	0.78	0.78	24.54	593.09	1.75	590.52

Table 4 – Multiple linear regression analysis of body weight from linear body measurements for female goats, CG = heart girth, BL = body length, TL = tail length, EL = ear length.

	I	β 1	β 2	β 3	R^2	A-R^2	CP	AIC	RSME	SBC
CG	-10.14	0.48			0.68	0.68	15.75	147.99	2.14	152.64
CG+BL	-13.47	0.38	0.18		0.71	0.70	5.81	138.82	2.02	145.81
CG+BL+TL	-11.57	0.35	0.19	0.13	0.72	0.71	3.61	121.42	1.99	132.44

Table 5 – Multiple linear regression analysis of body weight from linear body measurements for male goats, CG = heart girth, BL = body length, TL = tail length.

4. Conclusion

The multiple correspondence analysis showed that the goat population in the Estie, Simada, and Tach Gayint districts shares similar morphological characteristics. This might be due to the geographic proximity of the three districts. In all three districts, sex affected the BW, BL, CG, HW, HL, and RL, except for EL and TL. Male goats had consistently higher values than females for these quantitative traits. In the sample goat population, age was strongly influenced ($p<0.01$) by all measurements except TL and EL. The location of the animals had a significant effect on BW, BL, HW, HL, SC, and RL, except for CG, EL, and TL.

Ethics approval and consent to participate – All procedures involving animal use and care were closely monitored during the experimental period. The data collection protocols were reviewed and approved by the Research and Ethics Committee of the School of Animal and Range Science, Haramaya University (SARS 101/22, dated September 9, 2022), as they fulfill the required ethical standards stated in ICLAS (2012).

Variable name	Levels and descriptions	Variable name	Levels and descriptions
Coat Color Type	G1=White	Ear formation	N1=Erect
	G2=Red		N2=Pendulous
	G3=Gray		N3=Semi Pendulous
	G4=Black	Wattle	Z1=Present
	G5=White and red		Z2=Absent
	G6=White and brown	Ruff	X1=Present
	G7=All Color		X2=Absent
	G8=White and gray	Hair type	K1=Short and Smooth
	G9=Brown		K2=Long and Course
Coat Color Pattern	H1=Plain		K3=Short and Course
	H2=Spotted	Horn orientation	B1=Backward
	H3=Pied		B2=Obliquely Upward
Head Profile	M1=Concave	Horn shape	Y1=Straight
	M2=Straight		Y2=Curved
	M3=Slightly Concave		Y3=Scours

List of abbreviations.

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Authors' contributions – Habtamu Kegne and Michael Abera contributed to the design of the study, data collection, analysis, and manuscript writing. Moreover, Kefyalew Alemayehu participated in the study design, data analysis, and manuscript review.

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Data Availability statement – The data supporting the findings of this study are available upon request.

5. References

Abebe, T.et al. Soil and Land Use Characteristics in South Gondar Zone. Journal of Ethiopian Agricultural Sciences, 34(1), 55–68, 2024.

Abegaz, S.; Taye, M.; Tesema, Z. Phenotypic correlation and regression analysis of body measurements for weight prediction in indigenous goats of highland Ethiopia. Small Ruminant Research, 230, 107225, 2024. https://doi.org/10.1016/j.smallrumres.2024.107225

Alade, N. K.; Onakpa, M. M. Evaluation of linear body measurements as predictors of body weight in West African Dwarf goats. Livestock Research for Rural Development, 34(2). 2022. http://www.lrrd.org/lrrd34/2/nura34022.html

Al-Araimi N.A.; et al. Genetic Structure of Omani Goats Reveals Admixture Among Populations from Geographically Proximal Sites. Small Rumin. Res. 178:1–6, 2019.

Ashwini, J.P.; et al. Prediction of body weight based on body measurements in crossbred cattle. Int J Curr Microbiol Appl Sci. 8(3): 1597-1611, 2019.

Belete, T.; Getinet, M.; Solomon, A.; Tassew, M. Phenotypic Characterization of Indigenous Goat Population in Selected Districts of South Gondar Zone, Northern Ethiopia. East African Journal of Veterinary and Animal Sciences, Volume 6 (1): 31-44, 2022.

Birara, T.; Aberra, M.; Simret, B. Characterization of the Indigenous Goat Populations of South Gonder Based on their Morphometric Traits and Body Indices. Ethiop. J. Agric. Sci. 31(4) 71-87, 2021.

Central Statistics Agency (CSA), Agricultural Sample survey volume II: Report on Livestock and Livestock characteristics in Ethiopia. Statistical Bulletin 505, Addis Ababa, Ethiopia, 2022.

Central Statistics Agency (CSA). Federal Democratic Republic of Ethiopia, Agricultural sample survey. Report on livestock and livestock characteristics.; Statistical Bulletin No.583, Vol . 2, Addis Ababa, Ethiopia, 9-16, 2016.

CSA. Agricultural Sample Survey 2023/24. Central Statistical Agency of Ethiopia, 2024.

Duguma, G.; Terefe, E.; Tucho, T. Morphological and performance characteristics of indigenous goats in different agro-ecological zones of southern Ethiopia. Heliyon, 8(3), (2022). https://doi.org/10.1016/j.heliyon.2022.e09098

FAO. Phenotypic characterization of animal genetic resources. FAO Animal Production and Health Guidelines, Rome, Italy: Food and Agriculture Organization of the United Nations. No. 11, 2012.

Getachew, F.; Gizaw, S.; Kebede, K.; van Arendonk, J. A. M. Phenotypic and genetic characterization of indigenous goats under different production systems in Ethiopia. Tropical Animal Health and Production, 54(1), 1-11, 2022. https://doi.org/10.1007/s11250-021-02877-1

Gichohi-Wainaina, W. N.; Bett, R. C.; Ojango, J. M. K. Phenotypic characterization and production performance of small ruminants in tropical smallholder systems. Tropical Animal Health and Production, 55(2), 1–12, 2023. https://doi.org/10.1007/s11250-023-03423-7

Gizaw, S.; Tegegne, A.; Gebremedhin, B.; Hoekstra, D. Harnessing genetic resources for improved sheep and goat productivity in Ethiopia. Nairobi, Kenya: International Livestock Research Institute. ILRI Working Paper 22, 2011.

Grum, G. Community-Based Participatory Characterization of the Short-Eared Somali Goat Population Around Dire Dawa. An MSc thesis submitted to the School of Animal and Range Science, School of Graduate Studies, Haramaya University, 129, 2010.

Guang-Xin, E. et al. Genetic Diversity of the Chinese Goat in the Littoral Zone of the Yangtze River as Assessed by Microsatellite and mtDNA. Ecol. Evol. 8:5111–5123, 2018. Doi: 10.1002/ece3.4100.

Hagos, T.; Tegegne, F.; Gebremedhin, G. Body weight and linear measurements of indigenous goats in Tigray, Northern Ethiopia. Ethiopian Journal of Animal Production, 22(1), 45–57, 2022.

Halima, H.; Michael, B.; Barbara, R.; Markos, T. Phenotypic characterization of Ethiopian indigenous goat populations. African Journal of Biotechnology 11(73), 13838-13846, 2012.

Hassen, H.; Gizaw, S.; Dessie, T. Characterization and conservation of indigenous animal genetic resources in Ethiopia. In: State of Ethiopia's Animal Genetic Resources. ILRI, 2020.

Hlokoe, V.R.; Tyasi, T.L. Nguni cattle body weight estimation using regression analysis. Journal of Animal Health and Production, 10(3), pp.375-380, 2022.

Institute of Biodiversity Conservation (IBC). The State of Ethiopia's Farm Animal Genetic Resources: Country report. A Contribution to the First Report on the State of the World's Animal Genetic Resources, Addis Ababa, Ethiopia, 2004.

International Livestock Research Institute (ILRI). Livestock Production and Marketing in Ethiopia, 2011.

International Organizations Council for Laboratory Animal Science (ICLAS). International Guiding Principles for Research Involving Animals. Available online: http://Grants.Nih.Gov/ Grants/Olaw/Guiding_Principles_2012.Pdf (accessed on 4 August 2019), 2012.

LPPS, Koehler, R.I. Indigenous breeds, local communities. Documenting animal breeds and breeding from a community perspective. Lokhit Pashu- Palak Sansthan, Sadri, India. 80, 2005.

Mahilet, D. Characterization of Hararghe highland goat and their production system in eastern Hararghe. MSc Thesis, Haramaya University. 89, 2012.

MoA. Food Security and Livelihoods Assessment Report. Ministry of Agriculture, Ethiopia, 2023.

NMA. Climate Data and Agroecological Zonation Report. National Meteorological Agency of Ethiopia. (2023).

Odadi WO. Using heart girth to estimate live weight of heifers (Bos indicus) in pastoral rangelands of northern Kenya. Livest. Res. Rural. Dev. 30 (1): 2018.

Okpeku, M. et al. Application of Multivariate Principal Component Analysis to Morphological Characterization of Indigenous Goats in Southern Nigeria. Acta Agraria Slovenica, 98 (2), 2011.

Seyed, M.V.F.et al. Investigation of the genetic diversity of domestic Capra hircus breeds reared within an early goat domestication area in Iran. Genetics Selection Evolution. Pp 46:27, 2014.

South Gondar Administrative Zone Office of Agriculture (SGADO). South Gondar Administrative Zone Office of Agriculture and Rural Development Annual Report (unpublished), 2024.

Statistical Analysis System (SAS). SAS for Windows, Release 9.2, SAS Institute Inc., 2008.

Tadesse, Y.; Hassen, H.; Zewdu, W. Phenotypic characterization and production system of indigenous goat types in Ethiopia: Implications for conservation and genetic improvement. Livestock Research for Rural Development, 35(2), 2023.

Tesfaye, G.; Mekasha, Y.; Abegaz, S. Morphometric characterization and live body weight prediction in indigenous goat populations of Ethiopia. Tropical Animal Health and Production, 55(6), 452, 2023. https://doi.org/10.1007/s11250-023-04178-2

Tesfaye, K.; Tsegaye, Y.; Assefa, D. Phenotypic characterization of indigenous goats in Northwestern Ethiopia. Tropical Animal Health and Production, 53(5), 1–10, 2021. https://doi.org/10.1007/s11250-021-02747-2

Tesfaye, Y.; Mulugeta, A.; Tsegaye, Y. Characterization of indigenous goats and their production system in West Hararghe, Oromia region, Ethiopia. Journal of Animal and Feed Research, 11(3), 83–91, 2021. https://doi.org/10.51227/jafr2021.11.083

Tsegaye, D.; Assefa, G.; Abebe, T. Phenotypic characterization and production system analysis of indigenous goats in South Gondar Zone, Ethiopia. Tropical Animal Health and Production, 56(3), 145, 2024. https://doi.org/10.1007/s11250-024-04045-x

Wilson, R.T.; Durkin, J.W. Small ruminant production in central Mali: reproductive Performance in traditionally managed goats and sheep. Livestock Production Sciences, 19: 523–529, 1988

Yakubu, A. Path coefficient and path analysis of body weight and biometric traits in Yankasa lambs. Slovak Journal of Animal Science, 43: 17–25, 2010.

Yimer, N.; Abegaz, S.; Tadesse, M. Morphological characterization of indigenous goat types under traditional management systems in Ethiopia. Small Ruminant Research, 222, 2023. https://doi.org/10.1016/j.smallrumres.2023.106903.

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Emerging Fungal Pathogens in Wildlife: a systematic review of Veterinary Implications and One Health Challenges

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Abstract: Emerging fungal pathogens represent an increasing threat to global biodiversity, animal health, and public health, particularly in wildlife populations, where outbreaks may result in ecological, veterinary, and zoonotic consequences. This systematic review aims to identify the main emerging fungal pathogens affecting wildlife and to evaluate the available evidence regarding their veterinary, environmental, and human health implications within a One Health framework. A comprehensive literature search was conducted across PubMed, Scopus, Web of Science, and CAB Abstracts for studies published between 2000 and 2025, using predefined keywords related to emerging fungal pathogens, wildlife mycoses, zoonotic fungi, and One Health. A total of 147 records were identified. After duplicate removal and relevance screening, 72 articles underwent full-text evaluation, and 48 studies met the eligibility criteria and were included in the qualitative synthesis. The most frequently reported pathogens included *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* in amphibians, *Pseudogymnoascus destructans* in bats, and *Paranannizziopsis* spp. in reptiles. Reports originated from North America, Europe, South America, Asia, and Oceania, affecting amphibians, bats, reptiles, birds, and small mammals. Diagnostic confirmation was commonly based on molecular assays and histopathology, given nonspecific clinical signs. From a One Health perspective, several wildlife-associated fungi show documented or potential relevance to human health, particularly among immunocompromised individuals and professionals with occupational exposure. Human cases, when reported, were diagnosed using culture, molecular methods, and histopathology. Environmental drivers such as climate change, habitat disturbance, and anthropogenic dissemination were consistently associated with pathogen emergence and spillover risk.
Keywords: emerging mycoses, wildlife health, One Health, veterinary diagnostics, fungal pathogens.

1. Introduction

Fungal pathogens have historically received less attention than bacterial or viral agents in both veterinary and public health contexts. However, in recent decades, the emergence and re-emergence of fungal diseases in wildlife populations have gained increasing recognition as significant threats to biodiversity, ecosystem stability, and animal health worldwide (FISHER *et al.*, 2020; FISHER *et al.*, 2022). These pathogens often cause high-mortality events, contribute to severe population declines, and, in some cases, drive species toward extinction. Unlike many bacterial or viral diseases, fungal pathogens frequently persist in the environment for extended periods, complicating eradication efforts and facilitating recurrent outbreaks (FISHER *et al.*, 2022).

Several high-impact fungal diseases illustrate the scale and complexity of this problem. The chytrid fungi *Batrachochytrium dendrobatidis* (Bd) and *B. salamandrivorans* have devastated amphibian populations globally, contributing to the decline of more than 500 species and the extinction of at least 90 since the 1970s (SCHEELE *et al.*, 2019; MARTEL *et al.*, 2013). Similarly, *Pseudogymnoascus destructans*, the causative agent of white-nose syndrome in bats, has killed millions of hibernating bats across North America, with cascading ecological consequences due to their role in insect population control (FRICK *et al.*, 2017). In reptiles, *Ophidiomyces ophiodiicola*, *Nannizziopsis spp.*, and *Paranannizziopsis spp.* have emerged as significant causes of mycotic dermatitis, threatening both free-ranging and captive populations and raising concerns about cross-species transmission (SIGLER; HAMBLETON; PARÉ, 2013; LORCH *et al.*, 2016).

A complex interplay of ecological, climatic, and anthropogenic factors drives the emergence of fungal pathogens in wildlife. Climate change alters temperature and humidity patterns, creating new niches conducive to fungal proliferation (Garcia-Solache and Casadevall, 2010). Human activities, including global trade in wildlife and exotic pets, habitat fragmentation, and translocation of animals, further facilitate the spread of pathogenic fungi beyond their original geographic ranges (Fisher et al., 2020). Moreover, shifts in host–pathogen dynamics caused by environmental stressors can compromise wildlife immune responses, increasing susceptibility to infections (Voyles et al., 2018).

From a veterinary perspective, emerging fungal pathogens pose unique diagnostic and management challenges. Clinical manifestations in wildlife are often nonspecific or subclinical, complicating early detection and surveillance (UGOCHUKWU *et al.*, 2022). Accurate diagnosis typically requires histopathology, culture, and molecular tools such as PCR and sequencing, which may not be readily available in field settings (LORCH *et al.*, 2016). Furthermore, therapeutic options for free-ranging wildlife are limited, and antifungal resistance in some emerging pathogens poses significant challenges for effective disease control (FISHER *et al.*, 2022; WIEDERHOLD, 2023).

The One Health framework emphasizes the interconnectedness of human, animal, and environmental health. Many wildlife-associated fungi have zoonotic potential or can indirectly affect human health through ecological disruption. For example, declines in insectivorous bats caused by *P. destructans* can increase agricultural pest burdens, thereby impacting food security (Frick et al., 2017). Similarly, disruptions in amphibian communities can alter aquatic ecosystems, with cascading consequences for biodiversity and ecosystem services (Scheele et al., 2019).

Therefore, the overall objective of this systematic review is to synthesize current evidence on emerging fungal pathogens affecting wildlife, with a particular focus on their veterinary implications, diagnostic challenges, environmental drivers, and relevance within a One Health framework, integrating impacts on animal, human, and ecosystem health.

2. Development

2.1. Type of study

This is a qualitative narrative review that compiles and critically analyzes scientific evidence on emerging fungal pathogens affecting wildlife from January 2000 to October 2025. The study aimed to describe the diversity, epidemiology, diagnostic approaches, host–pathogen interactions, antifungal resistance, and One Health implications associated with these pathogens. For the study design, the recommendations of the PRISMA 2020 guidelines were adopted and adapted, as described by Page et al. (2021) and complemented by the methodological principles outlined in Haddaway et al. (2022) and Peters et al. (2020), to ensure greater transparency, reproducibility, and methodological rigor in the review process and the results obtained.

2.2. Descriptors and Database

An active literature search was conducted in the Scientific Electronic Library Online (SciELO), PubMed (National Library of Medicine), Virtual Health Library (VHL), Scopus, and Web of Science. Articles published in English, Spanish, and Portuguese between January 2000 and October 2025 were considered. The search used descriptors and keywords in English, Spanish, and Portuguese, including Fungal Pathogens, Wildlife, Veterinary Medicine, and One Health, combined with specific pathogen names such as B. dendrobatidis, *P. destructans, O. ophiodiicola, Nannizziopsis spp.,* and *Paranannizziopsis spp*. Descriptors were combined using the Boolean operator "AND", which restricted the results to documents containing all specified.

The inclusion criteria considered peer-reviewed articles published between January 2000 and October 2025 in English, Spanish, or Portuguese that addressed emerging fungal pathogens in wildlife and their relevance to veterinary medicine and One Health. Eligible studies focused on topics such as epidemiology, ecology, diagnostic approaches (e.g., histopathology, fungal culture, PCR, sequencing, MALDI-TOF), host–pathogen interactions, antifungal resistance, and disease management strategies. Original research papers, case reports, surveillance studies, and review articles relevant to these themes were included. The exclusion criteria eliminated articles that focused exclusively on human or plant pathogens without relevance to wildlife, lacked sufficient methodological detail or data for interpretation, or were not peer-reviewed. Abstracts, theses, editorials, and opinion papers were also excluded from the final synthesis.

2.3. Search Strategy

The literature search strategy was designed to ensure a comprehensive and systematic identification of relevant studies on emerging fungal pathogens in wildlife. A total of 147 records were initially identified across the selected databases (SciELO, PubMed, Virtual Health Library, Scopus, and Web of Science) using predefined descriptors and Boolean combinations. Following the removal of duplicate records and the application of inclusion criteria based on topical relevance, 72 articles were retained for full-text review. These articles were then subjected to a detailed screening process to assess methodological quality, data completeness, and alignment with the scope of this review. After this critical evaluation, 48 studies met all eligibility criteria and were included in the final qualitative synthesis. The selection process followed PRISMA 2020 guidelines (PAGE et al., 2021).

3. Summary of Results

The search results from the selected databases were organized into a PRISMA flowchart. After independent evaluation by two reviewers, the studies that met the inclusion criteria were categorized according to the type of data reported, including pathogen species, host taxa, geographic distribution, diagnostic approaches, and disease outcomes. The following key aspects were systematically documented: year of publication, authors, host species, host country, and identified fungal pathogens.

Additionally, figures were created to illustrate the distribution of annual scientific production on emerging fungal pathogens at both global and regional levels, providing a more straightforward overview of research trends and growing scientific interest in this field over time. Comparative analyses were also conducted to highlight variations in the diversity of reported pathogens across different taxonomic groups (amphibians, bats, reptiles) and their geographic occurrence.

3.1. Active Search Flowchart

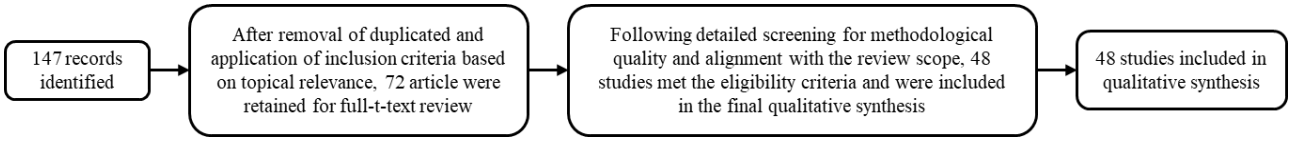

Figure 1 – Systematic Review Flowchart of Study Selection.

3.2. Geographic distribution of emerging fungal diseases in wildlife

The analysis of the 48 studies included in this qualitative narrative review demonstrates that research on emerging fungal diseases in wildlife is heavily concentrated in temperate regions of the Northern Hemisphere, particularly in Europe and North America, where the main surveillance networks and long-term monitoring programs are established (Fisher et al., 2012; Gibb et al., 2020).

Among these studies, 22 had a global scope; six were conducted in Europe, nine in North America, two involved collaborations between North America and Europe, and individual studies were conducted in Panama and Australia. Collectively, studies with a global scope discussed the emergence of these pathogens, including their ecological and health impacts, interactions with climate change, and implications within the One Health framework (Fisher et al., 2020; Fisher et al., 2024). In Europe, most studies reported infections by *Ophidiomyces ophiodiicola* in wild snakes (Franklinos et al., 2017; Müller et al., 2018) and chytridiomycosis outbreaks caused by *Batrachochytrium salamandrivorans* in amphibians (Martel et al., 2013; Martel et al., 2014), as well as transcontinental dissemination routes between Europe and Asia (Martel et al., 2020). A geographic heat map summarizing study locations and transcontinental collaborations is presented in Figure 2.

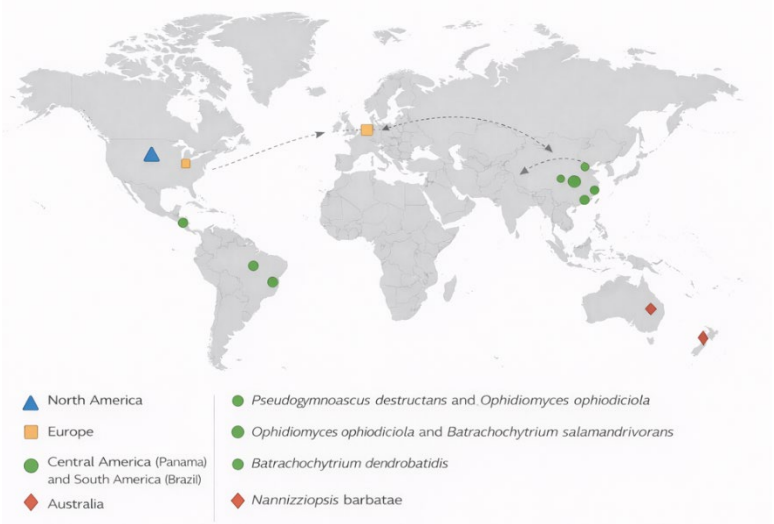

Figure 2 – Geographic distribution of studies on emerging fungal diseases in wildlife. The map summarizes the regional focus of published studies included in this review, highlighting Europe and North America as the main centers of research activity. Symbols indicate the primary fungal pathogens investigated in each region, including *Ophidiomyces ophiodiicola* and *Batrachochytrium salamandrivorans* in Europe, Pseudogymnoascus destructans and *O. ophiodiicola* in North America, *Batrachochytrium dendrobatidis* in Panama and Brazil, and *Nannizziopsis barbatae* in Australia. Dashed arrows indicate documented transcontinental dissemination routes between Europe and Asia. The map reflects study locations rather than pathogen prevalence.

In North America, most research focused on two ecologically significant fungal diseases: white-nose syndrome, caused by *Pseudogymnoascus destructans* in bat populations (Blehert et al., 2009; Frick et al., 2010), and ophidiomycosis in wild snakes associated with *O. ophiodiicola* (Allender et al., 2015; Lorch et al., 2016). These infections were shown to significantly affect host survival, reproductive success, and movement patterns. Across studies, molecular diagnostic approaches evolved, with earlier investigations relying primarily on histopathology and culture-based methods, and more recent studies incorporating DNA-based techniques such as conventional PCR, nested PCR, and quantitative real-time PCR targeting fungal ribosomal regions (e.g., ITS and 28S rRNA). These methods substantially improved pathogen detection and specificity. In addition, high-throughput approaches, including metagenomic sequencing, were applied in a subset of studies to characterize host-associated microbial communities and to explore environmental factors influencing pathogen persistence and transmission dynamics (Langwig et al., 2017; Davy et al., 2018).

In Central America, one study conducted in Panama documented shifts in disease dynamics within tropical amphibian assemblages affected by *B. dendrobatidis*, demonstrating changes in community composition and differential species susceptibility following pathogen emergence (Voyles et al., 2018). In Australia, one study reported the emergence of *N. barbatae* in free-living reptiles, affecting multiple lizard species across different regions and establishing this pathogen as an emerging threat at a continental scale rather than a localized or host-specific event (Boyle et al., 2020).

In the Southern Hemisphere, research remains limited and geographically uneven. In South America, Brazil is currently the only country with confirmed published records (n = 3 studies) documenting emerging fungal pathogens in free-living wildlife, specifically *Batrachochytrium dendrobatidis* (Bd) in amphibian populations. No eligible studies from other South American countries met the inclusion criteria of this review. Although fungal diseases affecting wildlife were reported before 2000, these early accounts were predominantly descriptive and lacked standardized surveillance frameworks or molecular diagnostic confirmation. Consequently, the increase in documented cases after 2000 is best interpreted as reflecting methodological advances, expanded surveillance efforts, and increased scientific awareness, rather than a true absence of emerging fungal pathogens in earlier periods. Overall, these results highlight a marked geographical bias in current research efforts and emphasize the need to strengthen surveillance and diagnostic programs in biodiversity-rich tropical regions that remain underrepresented in the global literature.

3.3. Brazil in the Global Context of Wildlife Fungal Diseases

Brazil represents a key region for understanding the distribution, diversity, and ecological impact of emerging fungal pathogens in wildlife. As summarized in Table 1, multiple studies have documented the presence of *B. dendrobatidis* across several Brazilian biomes (the Atlantic Forest, Caatinga, and lowland Amazon Forest). This is the pathogen's broad ecological range and persistence in tropical and subtropical ecosystems (Jenkinson et al., 2016; Carvalho et al., 2017; Benício et al., 2020; Lambertini et al., 2022). These studies reveal that *Bd* has likely been present in Brazil for nearly a century, with the earliest evidence dating back to the 1930s in preserved amphibian specimens, suggesting that chytridiomycosis may have contributed to amphibian population declines long before it was recognized as a global threat (Rodriguez et al., 2014; Fisher et al., 2012).

Moreover, molecular analyses based on PCR amplification and sequencing of fungal DNA, including multilocus genotyping approaches, have demonstrated the coexistence of a unique endemic lineage (Bd-Brazil) and a globally distributed lineage (Bd-GPL), indicating both long-term historical persistence and potential introductions from external sources. Despite this growing body of evidence, significant knowledge gaps remain, as no confirmed detections of *Batrachochytrium salamandrivorans*, *Ophidiomyces ophiodiicola*, or fungi of the genera *Nannizziopsis* and *Paranannizziopsis* have been reported in Brazilian wildlife to date (Waddle et al., 2020; Basanta et al., 2022).

This absence underscores the urgent need for expanded surveillance programs, improved diagnostic capacity, and targeted ecological studies. Given Brazil's exceptional biodiversity and complex ecosystems, addressing these gaps is crucial to advancing global understanding of fungal disease dynamics and strengthening wildlife conservation efforts.

Reference	Biome / Region	Location	Key Findings/Species
Jenkinson et al., 2016 (*Mol. Ecol.*)	Atlantic Forest (Southeast)	São Paulo, Paraná, Santa Catarina	Coexistence of endemic (*Bd-Brazil*) and globally distributed (*Bd-GPL*) lineages; evidence of local diversification and historical persistence.
Carvalho et al., 2017 (*Proc Royal Soc. B*)	Atlantic Forest (Southeast)	Various states in SE Brazil	Historical amphibian declines in Brazil linked to chytridiomycosis since mid-20th century.
Benício et al., 2020 (*Trop. Conserv. Sci.*)	Caatinga (Northeast)	Pernambuco, Paraíba	First evidence of high Bd prevalence in semi-arid species (*Rhinella granulosa*, *R. jimi*).
Lambertini et al., 2022 (*Sci. Rep.*)	Amazon (Lowland)	Pará, Amazonas	Detection of Bd in 57 amphibian species by qPCR and experimental infection.
Carvalho et al., 2017 (*Proc. Royal Soc. B*)	Atlantic Forest (Southeast)	Museum collections	Historical records of Bd dating back to the 1930s in preserved amphibian specimens.

Table 1 – Documented studies of *Batrachochytrium dendrobatidis* (Bd) in wild animals in Brazil.

3.4. Temporal Trends in Research on Emerging Fungal Pathogens in Wildlife (2012–2024)

The temporal distribution of the 48 studies analyzed in this review (Table 2) demonstrates a clear expansion of research on emerging fungal pathogens in wildlife over the past decade, reflecting shifting scientific priorities, improved diagnostic capacity, and increasing awareness of fungal threats. Although the literature search covered the period from 2000 to 2025, all eligible studies were published between 2012 and 2024. This pattern highlights that substantial scientific attention to fungal diseases in wildlife emerged primarily in the last decade, coinciding with their recognition as major drivers of biodiversity loss and significant challenges within the One Health framework (Fisher et al., 2012; Fisher et al., 2020).

Period	Number of Studies	Key Focus and Milestones	Representative References
2012–2015 – Foundational discoveries and early frameworks	12	Identification of key pathogens (*Batrachochytrium dendrobatidis*, *B. salamandrivorans*, *Ophidiomyces ophiodiicola*, *Pseudogymnoascus destructans*); development of conceptual models; recognition of wildlife trade as a driver of pathogen spread.	Fisher et al., 2012; Martel et al., 2013; Lorch et al., 2015; Frick et al., 2015; Fisher et al., 2015
2016–2019 – Expansion of taxonomic scope and geographic range	14	Inclusion of reptiles and bats; documentation of transcontinental spread; refinement of diagnostic and histopathology standards; focus on environmental and thermal ecology influences on disease dynamics.	Martel et al., 2016; Franklinos et al., 2017; Langwig et al., 2017; Voyles et al., 2018; Lorch et al., 2016
2020–2024 – Methodological	22	Adoption of environmental DNA (eDNA) detection; development of point-of-care assays; study of host	Walker et al., 2022; Woodhams et al., 2020;

Period	Number of Studies	Key Focus and Milestones	Representative References
innovation and One Health integration		microbiome interactions; research on antifungal resistance; establishment of surveillance networks; integration of One Health approaches.	Basanta et al., 2023; Thomas et al., 2024; Fisher et al., 2024

Table 2 – Temporal distribution of studies on emerging fungal pathogens in wildlife from 2012 to 2024.

Subsequent research expanded to include wildlife as a major driver of pathogen spread, documenting the emergence of additional fungal diseases across diverse taxa and ecosystems (Fisher et al., 2015). But how severe were these impacts? In several affected species, population declines exceeded 70–90%, with some local extinctions reported within a few years of pathogen introduction. For example, snake fungal disease caused by *O. ophiodiicola* has been associated with significant reductions in survival, body condition, and recruitment in wild snake populations. In contrast, white-nose syndrome caused by *Pseudogymnoascus destructans* has resulted in the loss of millions of bats across North America, with mortality rates surpassing 90% in some hibernacula. These dramatic declines illustrate not only how widely these pathogens have spread, but also the profound ecological consequences of fungal epizootics in wildlife populations.

Between 2016 and 2019, research diversified geographically and taxonomically, expanding beyond amphibians to include reptiles and bats. Studies during this period advanced understanding of *Ophidiomyces ophiodiicola* infections by describing lesion progression, host inflammatory responses, and impacts on survival in wild snakes (Lorch et al., 2016; Franklinos et al., 2017). The transcontinental spread of *B. salamandrivorans* was also documented, highlighting the role of wildlife trade in pathogen dissemination (Martel et al., 2016). Research further clarified how climate and thermal ecology influence disease dynamics by showing that temperature-dependent fungal growth, host thermoregulatory behavior, and seasonal conditions affect infection intensity, pathogen persistence, and transmission risk (Lorch et al., 2016; Franklinos et al., 2017). Advances in histopathology have improved the identification of diagnostic skin lesions, while molecular diagnostic approaches such as species-specific PCR and quantitative PCR have enhanced pathogen detection and surveillance capacity (Lorch et al., 2016; Franklinos et al., 2017).

The most recent phase (2020–2024) was marked by rapid methodological innovation and a shift toward predictive and applied research. Environmental DNA (eDNA) methods (Walker et al., 2022), point-of-care diagnostics (Thomas et al., 2024), and studies on host microbiomes (Woodhams et al., 2020) advanced the field, while antifungal resistance, cross-species transmission, and surveillance design became central topics.

3.5. Molecular and Genomic Insights into Emerging Fungal Pathogens

Molecular and genomic approaches have provided critical insights into the evolution, pathogenicity, and spread of emerging fungal pathogens in wildlife. Genomic studies on *Batrachochytrium dendrobatidis* revealed the coexistence of endemic and globally distributed lineages in Brazil, highlighting both historical persistence and multiple introductions (Jenkinson et al., 2016). Comparative genomics and population-level analyses have also elucidated the evolutionary origins and virulence determinants of *P. destructans* in bats and *O. ophiodiicola* in snakes (Lorch et al., 2016; Franklinos et al., 2017). eDNA and metagenomic tools further enhance surveillance by enabling direct detection of fungal DNA in soil and water samples, facilitating early outbreak detection without laboratory culture (Walker et al., 2022). These molecular advancements are transforming wildlife disease research, improving diagnostic precision, and providing valuable data on transmission dynamics and adaptive evolution. These molecular advancements are transforming wildlife disease research by improving diagnostic accuracy and generating high-resolution data on transmission dynamics and pathogen evolution. For example, whole-genome sequencing has been used to trace the geographic spread and evolutionary diversification of *B. salamandrivorans*, thereby enabling researchers to identify introduction pathways, detect multiple lineages, and assess adaptive changes associated with host susceptibility and environmental conditions (Martel et al., 2014; Farrer et al., 2017).

3.6. Environmental Reservoirs and Transmission Pathways

Environmental reservoirs play a crucial role in the persistence and dissemination of emerging fungal pathogens. Species such as *B. dendrobatidis* and *P. destructans* can survive for extended periods in water, soil, and organic matter. Remaining infectious even in the absence of hosts through persistence within environmental biofilms that enhance survival and resistance to environmental stressors (Garcia-Solache and Casadevall, 2010). Studies have demonstrated that *Ophidiomyces ophiodiicola* persists in moist soil and organic substrates, contributing to the recurrence of ophidiomycosis outbreaks in snake populations (Lorch et al., 2016; Franklinos et al., 2017). Global wildlife trade and the movement of animals have been implicated in the transcontinental introduction of *B. salamandrivorans*, as documented in Europe and Asia (Martel et al., 2013; Martel et al., 2020). These findings underscore how abiotic reservoirs, environmental conditions, and anthropogenic activities collectively shape the distribution and transmission of fungal pathogens, with environmental biofilms acting as protective niches that enhance pathogen persistence and facilitate repeated exposure of wildlife hosts. For example, *B. dendrobatidis* has been shown to persist within aquatic biofilms, where biofilm-associated cells exhibit increased survival under fluctuating environmental conditions, reinforcing the importance of targeted biosecurity and habitat management strategies (Garcia-Solache and Casadevall, 2010; Johnson and Speare, 2005).

3.7. Host–Pathogen Interactions and Immune Responses

Host–pathogen dynamics in emerging mycoses are governed by intricate molecular interactions and immune mechanisms. Pathogens such as *B. dendrobatidis* employ a range of virulence factors to invade host tissues and evade immune defenses. In contrast, hosts mount innate responses, including the production of antimicrobial peptides and inflammatory activation (Fisher et al., 2020). Research in Panama revealed that amphibian population recovery from chytridiomycosis is often linked to shifts in host immune response rather than pathogen attenuation (Voyles et al., 2018). Additionally, the skin microbiota of amphibians has been shown to provide natural protection by producing antifungal metabolites, influencing susceptibility to *Bd* infection (Woodhams et al., 2020). Environmental stressors, co-infections, and genetic diversity further modulate disease outcomes, emphasizing the complexity of host–pathogen interactions and the need for integrative approaches to enhance host resilience and disease management.

3.8. Antifungal Resistance and Therapeutic Challenges

Antifungal resistance is an emerging obstacle in controlling fungal diseases in wildlife, reducing the efficacy of available treatments and complicating management efforts. Mechanisms such as mutations in target genes, efflux pump overexpression, biofilm formation, and changes in cell membrane composition have been described in several pathogenic fungi (Fisher et al., 2022; Hui et al., 2024). The widespread use of azoles in agriculture has contributed to the development of cross-resistance in environmental isolates, posing additional challenges for veterinary applications (Fisher et al., 2024). Treating free-ranging wildlife is often unfeasible due to logistical constraints, ecological concerns, and the absence of approved antifungal protocols. As a result, alternative strategies such as the use of beneficial microbial communities, environmental interventions, and novel antifungal compounds are under investigation (Wiederhold, 2023). Addressing resistance will require integrated surveillance, stewardship, and One Health–oriented strategies that combine environmental, veterinary, and public health efforts. Evidence of antifungal treatment failure has been reported in wildlife, including cases of chytridiomycosis in amphibians treated with itraconazole, in which therapeutic responses were transient or ineffective, and reinfection occurred following treatment cessation (Garner et al., 2009; Berger et al., 2010).

3.9. Predictive Modeling and Climate Change Scenarios

Predictive modeling offers valuable tools for anticipating the dynamics of emerging fungal pathogens under changing environmental conditions. Species distribution models have been applied to forecast the spread of *B. salamandrivorans* under future climate scenarios, informing targeted surveillance and conservation strategies (Grisnik et al., 2023). Similar approaches in bat populations have used microclimate data from hibernacula to predict the expansion and impact of *P. destructans* (McClure et al., 2022). Changes in temperature, humidity, and precipitation patterns driven by climate change are expected to expand the ecological niches of many fungal pathogens, increasing disease risk in new regions (Garcia-Solache and Casadevall, 2010). Integrating climatic data with host distribution and land-use information strengthens predictive accuracy, providing essential insights for early intervention, risk assessment, and adaptive management in wildlife disease control.

3.10. Policy, Surveillance, and One Health Implementation

Coordinated regional and international policy frameworks and robust surveillance systems are vital to mitigating the impacts of emerging fungal pathogens. European risk assessments for *B. salamandrivorans* have led to recommendations for stricter trade regulations, quarantine measures, and improved biosecurity protocols (EFSA, 2018). Integrated surveillance programs that combine environmental sampling, wildlife diagnostics, and genomic monitoring enhance early detection and rapid response capabilities (Woods et al., 2023). The One Health approach promotes collaboration among veterinary, public health, environmental, and agricultural sectors, facilitating data sharing and coordinated action (Fisher et al., 2024). Expanding diagnostic capacity and surveillance infrastructure in biodiversity-rich regions remains a global priority. Strengthening these measures is crucial not only for protecting wildlife populations but also for preventing ecological disruption and safeguarding public health.

3.11. Limitations of the Present Review

As with most narrative reviews, this work has certain inherent limitations that should be acknowledged while interpreting its findings. The synthesis presented here is based on studies published in English, Portuguese, and Spanish, which may limit the inclusion of relevant literature available in other languages. Additionally, the existing body of research on emerging fungal pathogens remains geographically biased, with a predominance of studies from Europe and North America, reflecting current surveillance efforts rather than gaps in this review's design (Fisher et al., 2020; Gibb et al., 2020). Moreover, variation in methodologies and diagnostic approaches among published studies can influence the comparability of reported data. These aspects highlight broader challenges in the field and underscore the importance of expanding research efforts, particularly in biodiversity-rich tropical regions. Despite these constraints, this review provides a comprehensive synthesis of current knowledge. It identifies critical directions for future research and contributes valuable insights into the One Health implications of emerging fungal pathogens in wildlife.

4. Conclusion

Emerging fungal pathogens represent a significant and growing threat to wildlife, biodiversity, and ecosystem stability, with far-reaching implications for veterinary medicine and One Health. This review highlights the complex interplay of ecological, climatic, and anthropogenic drivers that facilitate the emergence, persistence, and spread of these pathogens, as well as the diagnostic and therapeutic challenges they pose. Advances in molecular tools, genomic analyses, and predictive modeling have deepened our

understanding of pathogen diversity, transmission dynamics, and host–pathogen interactions, while emphasizing the importance of environmental reservoirs and changing climatic conditions in shaping disease outcomes. Despite geographical and methodological gaps in current research, the synthesis presented here underscores the crucial roles of surveillance, interdisciplinary collaboration, and integrative approaches in addressing the emergence of fungal diseases. By consolidating current scientific evidence, this work contributes to a broader understanding of fungal threats in wildlife. It provides a foundation for future efforts to safeguard animal health, preserve biodiversity, and protect ecosystem function.

5. Reference

Allender, M. C.; Raudabaugh, D. B.; Gleason, F. H.; Miller, A. N. The natural history, ecology, and epidemiology of *Ophidiomyces ophiodiicola* and its potential impact on free-ranging snake populations. Fungal Ecol., v. 17, p. 187–196, 2015.

Basanta, M. D.; Martel, A.; Pasmans, F. Emerging fungal pathogens in wildlife: current knowledge and future challenges. Front. Vet. Sci., v. 9, 891324, 2022.

Benício, R. A.; Toledo, L. F.; Becker, C. G.; Haddad, C. F. B. First evidence of high prevalence of *Batrachochytrium dendrobatidis* in semi-arid species. Trop. Conserv. Sci., v. 13, p. 1–9, 2020.

Blehert, D. S.; Hicks, A. C.; Behr, M. et al. Bat white-nose syndrome: an emerging fungal pathogen? Science, v. 323, n. 5911, p. 227, 2009.

Boyle, D. G. et al. Emergence of *Nannizziopsis barbatae* in free-living Australian reptiles. PLoS Pathog., v. 16, n. 11, e1008893, 2020.

Carvalho, T.; Becker, C. G.; Toledo, L. F. Historical amphibian declines in Brazil linked to chytridiomycosis. Proc. Biol. Sci., v. 284, n. 1853, 20162254, 2017.

Davy, C. M.; Donaldson, M. E.; Willis, C. K. R. White-nose syndrome is associated with increased replication of a naturally persisting coronavirus in bats. Biol. Lett., v. 14, n. 9, 20180490, 2018.

EFSA Panel on Animal Health and Welfare (AHAW). Risk of survival, establishment and spread of *Batrachochytrium salamandrivorans* (Bsal) in the EU. EFSA J., v. 16, n. 4, 5259, 2018.

Farrer, R. A. et al. Genomic innovations linked to infection strategies across emerging pathogenic chytrid fungi. Nat. Commun., v. 8, 14742, 2017.

Fisher, M. C.; Alastruey-Izquierdo, A.; Berman, J. et al. Tackling the emerging threat of antifungal resistance to human health. Nat. Rev. Microbiol., v. 20, p. 557–571, 2022.

Fisher, M. C.; Garner, T. W. J.; Walker, S. F. Global emergence of *Batrachochytrium dendrobatidis* and amphibian chytridiomycosis in space, time, and host. Annu. Rev. Microbiol., v. 64, p. 291–310, 2020.

Fisher, M. C.; Gurr, S. J.; Cuomo, C. A. et al. Threats posed by the fungal kingdom to humans, wildlife, and agriculture. mBio, v. 11, n. 3, e00449-20, 2020.

Fisher, M. C.; Gurr, S.; Gow, N. A. R. et al. Tackling emerging fungal threats to animal health, food security, and ecosystem resilience. Philos. Trans. R. Soc. B, v. 371, 20160332, 2016.

Fisher, M. C.; Henk, D. A.; Briggs, C. J. et al. Emerging fungal threats to animal, plant and ecosystem health. Nature, v. 484, n. 7393, p. 186–194, 2012.

Fisher, M. C. et al. A One Health roadmap towards understanding and mitigating emerging fungal antimicrobial resistance. npj Antimicrob. Resist., v. 2, n. 1, 2024.

Franklinos, L. H. V. et al. Emerging fungal pathogen *Ophidiomyces ophiodiicola* in wild European snakes. Sci. Rep., v. 7, 3844, 2017.

Frick, W. F.; Pollock, J. F.; Hicks, A. C. et al. An emerging disease causes regional population collapse of a common North American bat species. Science, v. 329, n. 5992, p. 679–682, 2010.

Frick, W. F.; Cheng, T. L.; Langwig, K. E. et al. Pathogen dynamics during invasion and establishment of white-nose syndrome explain mechanisms of host persistence. Ecology, v. 98, n. 3, p. 624–631, 2017.

Garcia-Solache, M. A.; Casadevall, A. Global warming will bring new fungal diseases for mammals. mBio, v. 1, n. 1, e00061-10, 2010.

Gibb, R. et al. Zoonotic host diversity increases in human-dominated ecosystems. Nature, v. 584, n. 7821, p. 398–402, 2020.

Grisnik, M.; Gray, M. J.; Piovia-Scott, J. et al. Incorporating caudate species susceptibilities and climate change into models of *Batrachochytrium salamandrivorans* risk in the United States. Biol. Conserv., v. 284, 110181, 2023.

Haddaway, N. R.; Page, M. J.; Pritchard, C. C.; McGuinness, L. A. PRISMA 2020: R package and ShinyApp for producing PRISMA 2020 compliant flow diagrams. Syst. Rev., v. 11, n. 1, 43, 2022.

Hui, M. et al. Emerging antifungal resistance: mechanisms and management strategies. Front. Cell. Infect. Microbiol., v. 14, 2024.

Jenkinson, T. S. et al. Amphibian-killing chytrid in Brazil comprises both locally endemic and globally expanding populations. Mol. Ecol., v. 25, n. 13, p. 2978–2996, 2016.

Lambertini, C. et al. Amphibian chytrid fungus in the Brazilian Amazon: prevalence, species richness and lineage diversity. Sci. Rep., v. 12, n. 1, 1489, 2022.

Langwig, K. E.; Frick, W. F.; Reynolds, R. et al. Host and pathogen ecology drive the seasonal dynamics of white-nose syndrome. Proc. R. Soc. B, v. 282, 20142335, 2015.

Lorch, J. M. et al. Snake fungal disease: an emerging threat to wild snakes. Philos. Trans. R. Soc. B, v. 371, 20150457, 2016.

Martel, A. et al. *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. Proc. Natl. Acad. Sci. U.S.A., v. 110, n. 38, p. 15325–15329, 2013.

Martel, A.; Blooi, M.; Adriaensen, C. et al. Recent introduction of a chytrid fungus endangers Western Palearctic salamanders. Science, v. 346, n. 6209, p. 630–631, 2014.

Martel, A.; Blooi, M.; Pasmans, F. Transcontinental spread of the emerging amphibian pathogen *Batrachochytrium salamandrivorans*. Emerg. Infect. Dis., v. 26, n. 9, p. 2122–2124, 2020.

McClure, M. L.; Hranac, C. R.; Haase, C. G. et al. Projecting the compound effects of climate change and white-nose syndrome on North American bat species. Clim. Change Ecol., v. 3, 100047, 2022.

Müller, L.; Martel, A.; Praet, S.; Pasmans, F. Ophidiomycosis in a European snake: case report and review of literature. Vet. Rec. Case Rep., v. 6, n. 4, e000730, 2018.

Page, M. J. et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ, v. 372, n. 71, 2021.

Peters, M. D. J. et al. Chapter 11: Scoping Reviews. In: Aromataris, E.; Munn, Z. (eds.). JBI Manual for Evidence Synthesis. Adelaide: JBI, 2020.

Rodriguez, D.; Becker, C. G.; Pupin, N. C.; Haddad, C. F. B.; Zamudio, K. R. Long-term endemism of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* in the Brazilian Atlantic Forest. Mol. Ecol., v. 23, n. 4, p. 774–787, 2014.

Scheele, B. C. et al. Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. Science, v. 363, n. 6434, p. 1459–1463, 2019.

Sigler, L.; Hambleton, S.; Paré, J. A. Molecular characterization of reptile pathogens currently known as members of the *Chrysosporium* anamorph of *Nannizziopsis vriesii* complex. Mycologia, v. 105, n. 2, p. 469–492, 2013.

Thomas, P. et al. Point-of-care diagnostics for emerging wildlife fungal pathogens. EcoHealth, v. 21, n. 1, p. 25–36, 2024.

Ugochukwu, E. I.; Osakwe, O. N.; Oragwa, A. O.; Nnadi, N. E.; Onyekwere, E. C. Emerging fungal diseases of wildlife: diagnostic challenges and control strategies. Vet. World, v. 15, n. 3, p. 563–573, 2022.

Voyles, J. et al. Shifts in disease dynamics in a tropical amphibian assemblage are not due to pathogen attenuation. Science, v. 359, n. 6383, p. 1517–1519, 2018.

Waddle, A. W.; Refsnider, J. M.; Adams, C. et al. Global patterns of emerging fungal pathogens in reptiles. Fungal Ecol., v. 47, 100956, 2020.

Walker, D. M.; Raudabaugh, D. B.; Miller, A. N. Environmental DNA as a tool for monitoring fungal pathogens. Fungal Ecol., v. 54, 101115, 2022.

Wiederhold, N. P. Antifungal resistance: current trends and future strategies to combat. Infect. Dis. Clin. N. Am., v. 37, n. 2, p. 271–288, 2023.

Woodhams, D. C. et al. Antifungal isolates database of amphibian skin-associated bacteria and function against emerging fungal pathogens. Ecology, v. 101, n. 1, e02918, 2020.

Woods, M.; Ashraf, N.; Bromley, M. et al. A One Health approach to overcoming fungal disease and antifungal resistance. WIREs Mech. Dis., v. 15, e1610, 2023.

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Preliminary study on ozone hemotherapy for canine ischemic renal failure

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Abstract: In dogs, kidney injury is a sudden deterioration in renal function that can be fatal. Ischemia, inflammation, nephrotoxin exposure, and infectious diseases are the most frequent causes. In several fields of regenerative medicine, ozone therapy has recently attracted considerable attention. Clinical studies have shown that ozone therapy is safe and effective, and that it reduces readmission rates for kidney injury in animal models of renal disorders. This study investigated the efficacy of ozone therapy in experimentally induced ischemic renal failure in dogs. Ten dogs were used in this study. All dogs underwent right nephrectomy and 60 minutes of left renal ischemia followed by reperfusion. The animals were divided into two equal groups. The control group received no treatment, and the O3 group received an ozone–oxygen mixture. All groups were evaluated clinically and biochemically until day 40 after surgery, after which they underwent histopathological assessment. Major ozonated autologous hemotherapy substantially reduced the degree of elevation in the treatment group's serum blood urea nitrogen and creatinine levels relative to the control group. Medical ozone therapy considerably reduced the degree of glomerular filtration rate decline and notable alterations; it resulted in a morphological improvement in renal tubular structure compared with that in the control group, and a well-structured improvement in glomeruli, which were reasonably close to those in the healthy control group. According to the findings, ozone therapy may be an effective way to enhance renal function. However, our results indicate that the aforementioned treatment has potential as a straightforward therapeutic strategy for the management of renal failure that may be caused by ischemia or other factors.
Keywords: Acute Renal Failure, Autohemotherapy, Dogs, Medical ozone, Nephrectomy.

1. Introduction

Renal diseases, which are common in dogs and often associated with a poor prognosis in the late stages, can cause abrupt declines in renal function. About 2%–5% of all dogs suffer from renal failure, one of the most dangerous issues in the canine population that can be gradually brought on by renal diseases (Kumar *et al*., 2023).

Ischemic renal failure occurs when there is a brief disruption and subsequent restoration of blood flow to the kidney. It frequently occurs in clinical settings and could include renal artery blockage, heart surgery, and renal transplantation (Shen *et al*., 2024). It causes nitrogenous wastes to be retained in plasma and a sharp decline in renal function. Dogs frequently contract the illness, which has a high morbidity and fatality rate (Cao *et al*., 2020). Renal failure has a highly complex pathophysiology that includes adenosine triphosphate (ATP) depletion, intracellular Ca+2 and reactive oxygen species buildup, proinflammatory cytokine release, and activation of the apoptotic pathway (Yang *et al*., 2023). Acute renal injury in dogs frequently necessitates extended hospital stays, which can be expensive, and they face the risk of acquiring chronic kidney disease (Chen *et al*., 2023).

Ozone has an antioxidant defense system and medicinal qualities that modulate apoptosis. Although many techniques can be used to administer medical ozone, major ozonated autohemotherapy is the most dependable and sophisticated method (Yang *et al*., 2023; Luo *et al*., 2023). Depending on the intended therapeutic goal, ozone therapy can be administered at doses ranging from 1 µg/mL to 100 µg/mL, with safety and efficacy, through various methods (Serra *et al*., 2023). O3 dose methods include topical and infiltrative therapy (for localized effects, such as musculoskeletal and germicidal), autohemotherapy, and rectal insufflation (for systemic effects), and they vary according to the objectives and site of therapy (Hidalgo-Tallón *et al*. 2022). Ozone therapy reduces oxidative stress and inflammation, which are implicated in organ damage in chronic illnesses (Lino *et al*., 2024).

Moreover, ozone increases the activity of enzymes such as hydrogen peroxide, oxidized glutathione reductase, and superoxide dismutase by efficiently eliminating free radicals, thereby enhancing local tissue metabolism, stimulating fibroblast proliferation, facilitating collagen fiber synthesis, and supporting angiogenesis (Wen and Chen, 2020). Ozone therapy facilitates the secretion of growth factors by macrophages and fibroblasts, promoting angiogenesis and granulation tissue formation, thereby accelerating tissue healing. The therapeutic mechanism of ozone can be attributed to the upregulation of growth factors, including vascular endothelial growth factor, transforming growth factor beta, and platelet-derived growth factor (Sun *et al*., 2024). It increases blood flow and oxygen transport in ischemic tissues (Emre *et al*., 2024). Xanthine oxidase is thought to mediate oxidative damage during renal ischemia. When cells are subjected to ischemia, this enzyme breaks down nucleotides.

Nitric oxide (NO), when supported by ozone therapy, can be a defense mechanism against endothelin-1-induced kidney damage, inflammation, and vasoconstriction (Delgadillo-Valero *et al*., 2023). Ozone never exceeds 5% of the gaseous mixture when therapeutic oxygen is used as the carrier; the highest ozone concentrations in this context are estimated at roughly 100 mg/L (Travagli *et al*., 2023). Ozone therapy works by boosting cellular metabolism, decreasing the production of proinflammatory prostaglandins and allogenic compounds, increasing the release of immunosuppressive cytokines, reducing oxidative stress by inducing the

synthesis of antioxidant enzymes, increasing the amount of oxygen that reaches tissues, and stimulating angiogenesis (Sumida *et al*., 2022). Ozonated blood can activate endothelial cells, causing them to release NO. As a well-known vasorelaxant, NO dilates vascular walls and exerts positive effects, especially in hypoxic tissues (Boczkowska *et al*., 2022). According to the study's hypothesis, ozone therapy may restore and enhance renal function and serve as a viable alternative solution to renal failure.

The use of medical ozone in the treatment of various kidney diseases has been the subject of recent clinical research. However, research on the use of medical ozone in the treatment of renal failure is scarce. Therefore, the purpose of this study is to evaluate the medical use of ozone in the regeneration and function of kidneys affected by ischemic renal failure.

2. Materials and Methods

2.1. Ethics Statement

The current study was approved by the Veterinary Medical College's Animal Ethics Committee at the University of Basrah (Approval No. 63/2024). Physical examination, complete blood count (CBC), and serum biochemical analysis were performed on all dogs to check for systemic diseases and confirm normal renal function. Each dog was vaccinated against rabies and given antiparasitic drugs. Food and drink were also readily available.

2.2. Experimental Design

Ten adult male mongrel dogs aged 1–1.5 years were used in this study. The body weights of the animals were extended from 18 kg to 22 kg, with a mean of 20 kg. The right kidneys of all dogs underwent nephrectomy, and the left kidneys were subjected to renal ischemia by clamping the renal artery and vein for 60 min. The animals were divided randomly into two equal groups as follows: Group I: The dogs were treated with an ozone/oxygen gas mixture administered via major ozonated autohemotherapy. Group II: The dogs received no treatment. All dogs were euthanized 40 days after surgery.

2.3. Surgical Procedure

Induction of unilateral renal ischemia reperfusion (I/R):

The procedure was implemented in a sterile environment. All animals were denied food for 10 h and allowed unrestricted access to water until 5 h before the procedure. Each dog received prophylactic antibiotic (pen-strep, 8 mg/kg) intramuscularly 1 h before surgery.

A combination of 2% xylazine hydrochloride at 5 mg/kg body weight (intramuscular injection [I/M]) and 10% ketamine hydrochloride at 10 mg/kg body weight (I/M) was used to anesthetize the dogs. To be ready for aseptic surgery, the dogs were placed in the dorsal position, and the surgical site, which runs from the xiphoid cartilage to the pubic bone, was cleaned and trimmed. An abdominal incision was made in the midline.

The bowels were retracted upon entry into the peritoneal cavity, and an atraumatic vascular clamp was used to cross-clamp the renal artery and vein of each dog's left kidney to cause ischemic injury. After 60 min, the clamp was released to allow for reperfusion.

Successful ischemia or reperfusion was assessed by tracking the change in tissue color from red to navy blue or from navy blue to red, respectively. Meanwhile, the right kidneys underwent nephrectomy.

During the surgical procedure, all dogs were administered intravenously with standard saline solution (10 mL/kg/h). The surgical wound was closed using polydioxanone (PDS II, size 2-0), followed by a continuous suture pattern. Then, the skin was closed with an interrupted suture pattern using a nylon suture (size 2-0). Throughout the planned study period, each animal was kept in a separate cage. In addition to daily medical care after surgery, the dogs were given injectable penicillin streptomycin for four days at doses of 10,000 IU and 20 mg/kg body weight. The dogs were then given unrestricted mobility. Fourteen days after the procedure, the wound sutures were removed. Every dog underwent clinical, biochemical, and histological evaluation (Cao *et al.,* 2020).

2.4. Biochemical Analysis

Creatinine (SCr) and blood urea nitrogen (BUN) levels were used to evaluate renal function. Five milliliters of blood were drawn from each dog's cephalic vein before surgery, two days after surgery, and every 10 days after treatment to ascertain the serum chemical profile. The serum BUN and SCr reference ranges were 8–30 and 0.5–1.5 mg/dl, respectively. SCr and serum BUN levels exceeding the standard limits were deemed abnormal (Lee *et al*., 2017).

2.5. Clinical Evaluation

All dogs were observed to evaluate the clinical signs on days 10, 20, 30, and 40 postoperatively.

2.6. Major Ozonated Autohemotherapy

Two days after surgery, each dog's cephalic vein yielded 25 mL of blood, which was then placed in a sterile transfusion blood bag with 13 mL of sodium citrate (3.8%, 380 mg/mL). The animal's weight was used to determine the blood volume (25 mL). The blood bag was filled with the oxygen–ozone mixture at a dose of 37.3 ug/mL. A generator with a production capacity of 0.00023 g/min, fueled by a 99.5% pure oxygen ampoule at a pressure of roughly 250 kgf/cm^2 and a flow rate of 3 L/min, produced the oxygen–ozone combination. The blood was reintroduced into the animal via the cephalic vein after ozonation and gentle shaking for 5 min, until the foam disappeared and the blood turned bright red (Sumida *et al*., 2023; Sancak *et al*., 2017).

2.7. Kidney Harvest

After 40 days of treatment, the dogs' left kidneys were promptly removed, and the dogs were euthanized. The renal tissue samples were preserved in 10% neutral-buffered formalin for histological analysis (Aum *et al*., 2023).

2.8. Histopathology Evaluation

On day 40 after surgery, renal tissue was taken from the left kidney's caudal pole, preserved in 10% buffered formalin, embedded in paraffin, and cut into sections that were 3 µm thick. The sections were stained with hematoxylin and eosin to assess histological alterations (Liu *et al*., 2023).

3. Statistical Analysis

The results were expressed as mean values–standard errors. Data were statistically analyzed using an independent-samples t-test and a one-way analysis of variance (ANOVA) with multiple-comparison tests in a statistical software program (SPSS for Windows, version 22, USA). Differences were considered significant at $P \leq 0.05$.

4. Results

4.1. Clinical Evaluation

The most common clinical signs that were observed postoperatively in all the dogs were lethargy, anorexia, vomiting, diarrhea, polyuria, and polydipsia.

Clinical symptoms observed after surgery occurred in all animals during the first 10 days after treatment, but they gradually began to disappear in the O3 group. In the control group, the signs continued until the 20th day, then appeared intermittently until they gradually disappeared after the 30th day.

4.2. Biochemical Evaluation

Major ozonated autohemotherapy notably reduced serum BUN and SCr elevations compared with the control group at 10, 20, 30, and 40 days after treatment in ischemic renal failure. The levels of urea in the O3 group on days 10, 20, 30, and 40 were 379,778, 200,262, 872, and 46,146 mg/dL, respectively, whereas those in the control group were 364,298, 310,302, 264,81, and 214,546 mg/dL, respectively. The levels of SCr in the O3 group on days 10, 20, 30, and 40 were 4.842, 2.91, 2.298, and 1.654 mg/dL, respectively, whereas those in the control group were 4.422, 3.91, 3.412, and 2,846 mg/dL, respectively, as shown in Figures 1 and 2 (Aum *et al*., 2022).

4.3. Histopathology Evaluation

The histopathological sections of the kidneys from the positive control group showed severe glomerular atrophy and cystic dilation of renal tubules (Figure 3). The O3 group showed marked glomerular structural and morphological improvements in renal tubules compared with the control group (Figure 4). The O3 group also showed remarkable changes, representing a highly structural improvement in glomeruli, approximating those of the healthy control, and a morphological improvement in renal tubules compared with the control group (Figure 5).

5. Discussion

Medical ozone is utilized in human and veterinary medicine despite its status as a pollutant. Ozone therapy, which involves the medicinal use of an ozone/oxygen gas mixture, is based on the idea that ozone quickly dissociates into water and releases a reactive form of oxygen that can oxidize cells. This dissociation increases the amount of ATP and oxygen available for cellular activity. Systemic and local routes are the two primary methods for delivering ozone. Ozone autohemotherapy, which involves *ex vivo* injection of a specific concentration of oxygen–ozone into a preset volume of blood, is used for systemic administration. Afterward, the patient receives this oxygenated-ozonated blood (Sciorsci *et al*., 2020).

Ozone therapy improves the body's antioxidant responses by causing regulated oxidative stress. However, little is known about how this treatment affects canine renal failure. Our goal was to evaluate the clinical, biochemical, and clinical characteristics of dogs affected with renal failure receiving autohemotherapy with oxygen and ozone (Oliveira *et al*., 2024). Ozone therapy's clinical trials are still in their infancy, despite preclinical research suggesting it may be effective for treating renal ailments. In accordance with Boczkowska-Radziwon *et al*. (2022), substantial ozonated autohemotherapy was used in this investigation because ozonated blood can activate endothelial cells via lipid peroxidation products and drive them to generate NO, which dilates the vessel walls and has positive effects, especially in hypoxic tissues.

Clinical symptoms, such as diarrhea, that resulted from direct gastrointestinal injury brought on by the presence of uremic toxins were observed in all groups in this study after the induction of ischemic renal failure. These symptoms are consistent with those reported by Rimer *et al.* (2022). Systemic hypertension and decreased medullary concentration capacity may be the cause of polyuria and polydipsia. Lack of appetite and reduced feed intake may cause anorexia and weakness, and intestinal malabsorption and uremic catabolism may induce weight loss and weakness. Moreover, uremic toxins may cause vomiting by stimulating the medullary emetic chemoreceptor trigger zone, leading to gastroenteritis, as explained by Pathak *et al.* (2023).

The histopathological sections of the kidneys from the positive control group showed severe glomerular atrophy and cystic dilation of renal tubules, resulting from ischemic changes in the renal parenchyma. These results are consistent with those of Dunaevich *et al*. (2020), who reported that ischemia is a significant cause of acute renal failure. In the current study, renal failure

was induced via the occlusion of the renal blood supply for 60 min with atraumatic clamps. These results are also consistent with those of Fan *et al*. (2023), who explained that damage to the renal parenchyma, glomeruli, renal tubules, renal interstitium, and renal microvessels is the consequence of ischemic renal failure. Compared with the control group, the ozone group showed glomerular morphological improvement and mild renal tubular dilation, reflecting morphological recovery following ischemic changes in the renal parenchyma.

The ozone group showed marked structural improvement in glomeruli and morphological improvement in renal tubules compared with the control group, reflecting recovery from ischemic changes in the renal parenchyma. This result can be explained by the finding of Gan *et al*. (2023), who reported that ozone may freely diffuse into RBCs and has a strong antioxidative stress effect. Activating phosphofructokinase can increase ATP and 2,3-DPG levels, improve blood oxygen delivery to hypoxic tissues, and exert an anti-inflammatory effect by reducing the synthesis of inflammatory factors, such as TNF-α, IL-1β, and IL-6. Moreover, ozone helps treat ischemia-reperfusion injury in organs. The ozone group in the current study showed substantial changes, representing a significant structural improvement of the glomeruli, approaching those of the healthy kidney, and a morphological improvement of renal tubules compared with the control group, due to complete morphological recovery following ischemic changes in the renal parenchyma. These results confirm the effects of ozone therapy on kidney tissue during treatment and align with those of Sun *et al.* (2024), who reported that ozone plays a vital role in regulating cellular proliferation and accelerating tissue repair. It can also help macrophages and fibroblasts secrete growth factors that promote angiogenesis.

Our results also align with those of Sumida et al. (2022), who reported that ozone therapy stimulates angiogenesis, increases the release of immunosuppressive cytokines, decreases the production of proinflammatory prostaglandins, increases oxygen delivery to tissues, and activates cellular metabolism. Enhancing erythrocyte pliability and potentially reducing blood viscosity and erythrocyte aggregation improve tissue oxygenation and increase renal blood flow during reperfusion in an organ that has previously experienced inadequate blood flow. The ozone group in the current study was highly superior to the acute renal failure control group.

Ten, twenty, thirty, and forty days after treatment for ischemic renal failure, major ozonated autohemotherapy substantially decreased the levels of blood BUN and SCr compared with those in the control group. As previously mentioned, the benefits of ozone therapy led to this improvement in renal function, as evidenced by a decrease in BUN and SCr levels.

6. Conclusions

The results of our investigation indicate that ozonated hemotherapy has potential as a straightforward therapeutic strategy for the management of renal failure that may be caused by ischemia or other factors.

Our study has several limitations. First, the dogs were followed up for only 40 days. Long-term studies are needed to evaluate the effectiveness of ozone therapy in ischemic renal failure and to determine whether it can prevent progression to chronic kidney disease. Second, the dogs were administered a single fixed dose concurrently with ischemia. Repeated administration of such therapy may be synergistic.

Authors' Contributions: BKK, RMN, WMMS, and AAI – development of the methodology, preparation and writing of the initial draft, review and editing of the manuscript, and data analysis. BKK, RMN, and AAI – preparation of samples, surgical operations, and laboratory analysis. WMMS and AAI looked over the document, provided feedback, and approved the final version.

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7. References

Aum J, Jang MJ, Kim YS, et al. Comparison of Stromal Vascular Fraction and Adipose-Derived Stem Cells for Protection of Renal Function in a Rodent Model of Ischemic Acute Kidney Injury. Stem Cells Int, 2022(1); 1379680, 2022.

Boczkowska-Radziwon B, Olbromski P, Rogowska A, et al. Ozonation of Whole Blood Results in an Increased Release of Microparticles from Blood Cells. Biomolecules, 12:(2); 164, 2022.

Cao J, Lu X, Gao F, et al. Assessment of neutrophil gelatinase-associated lipocalin as an early biomarker for canine renal ischemia-reperfusion injury. Ann Transl Med J, 8:(22); 1491, 2021.

de Oliveira PL, Mendonça MO, Gonçalves GS, et al. Ozone therapy by rectal insufflation in dogs: safety and oxidative stress-a randomized crossover study. Vet Res Commun, 48:(4);2263-2280, 2024.

Delgadillo-Valero LF, Hernández-Cruz EY, Pedraza-Chaverri J. The protective role of ozone therapy in kidney disease: a review. Life, 10; 13:(3); 752, 2023.

Dunaevich A, Chen H, Musseri D, et al. Acute on chronic kidney disease in dogs: Etiology, clinical and clinicopathologic findings, prognostic markers, and survival. J V Int Med, 34:(6); 2507-2515, 2020.

Emre AN, Erol H, Balcıoğlu E, et al. Efficiency of ozone (O3) therapy on experimental acidic skin burns in rats. Veterinaria México OA, 11; 2024.

Fan M, Lan X, Wang Q, Shan M, Fang X, Zhang Y, Wu D, Luo H, Gao W, Zhu D. Renal function protection and the

mechanism of ginsenosides: Current progress and future perspectives. Front Pharmacol, 6:(14); 1070738, 2023.

Gan Y, Tian X, Yao H, et al. Ozone Improves Oxygenation and Offers Organ Protection after Autologous Blood Transfusion in a Simulated Carbon Dioxide Pneumoperitoneal Environment in a Rabbit Hemorrhagic Shock Model. Transfu Med Hemother, 51:(3); 165-75 2024.

Hidalgo-Tallón FJ, Torres-Morera LM, Baeza-Noci J, et al. Updated review on ozone therapy in pain medicine. Front physiol, 13: 840623, 2022.

Kwiatkowska E, Kwiatkowski S, Dziedziejko V, et al. Renal microcirculation injury as the main cause of ischemic acute kidney injury development. Biology. 12:(2); 327, 2023.

Lee SJ, Ryu MO, Seo MS, et al. Mesenchymal stem cells contribute to improvement of renal function in a canine kidney injury model. In Vivo, 31:(6); 1115-1124, 2017.

Lino VT, Marinho DS, Rodrigues NC, et al. Efficacy and safety of ozone therapy for knee osteoarthritis: an umbrella review of systematic reviews. Front Physiol, 15: 1348028, 2024.

Liu H, Huang L, Chen F, et al. Adipose-derived mesenchymal stem cells secrete extracellular vesicles: A potential cell-free therapy for canine renal ischaemia-reperfusion injury. Vet Med Science, 9:(3); 1134-1142, 2023.

Luo Y, Zhu J, Zhao F, et al. PIM1 attenuates renal ischemia–reperfusion injury by inhibiting ASK1-JNK/P38. International Immunopharmacology, 114; 109563, 2023.

Pathak SK, Tiwari A, Dubey A, et al. Clinical abnormalities pertaining to renal disorders in canines. Pharma Innovation 12:(5); 209-212, 2023.

Rimer D, Chen H, Bar-Nathan M, et al. Acute kidney injury in dogs: Etiology, clinical and clinicopathologic findings, prognostic markers, and outcome. J Vet Int Med, 36(2), 609-618, 2022.

Sancak EB, Turkön H, Çukur S, et al. Major ozonated autohemotherapy preconditioning ameliorates kidney ment – infrastructure – laboratories etc.

ischemia-reperfusion injury. Inflammation, 39:(1); 209-217, 2016.

Sciorsci RL, Lillo E, Occhiogrosso L, et al. Ozone therapy in veterinary medicine: A review. Res Vet Science, 130; 240-246, 2020.

Serra ME, Baeza-Noci J, Abdala CV, et al. Clinical effectiveness of medical ozone therapy in COVID-19: the evidence and gaps map. Med Gas Res, 13:(4); 172-180, 2023.

Shen J, Sun Y, Zhuang Q, et al. NAT10 promotes renal ischemia-reperfusion injury via activating NCOA4-mediated ferroptosis. Heliyon, 10:(2); 2024.

Sumida JM, Hayashi AM. Ozone therapy in veterinary medicine: clinical indications and techniques. Acta Veterinaria Brasilica, 16:(4); 294-304, 2022.

Sun H, Heng H, Liu X, et al. Evaluation of the healing potential of short-term ozone therapy for the treatment of diabetic foot ulcers. Front Endocrinol, 14; 1304034, 2024.

Travagli V, Iorio EL. The biological and molecular action of ozone and its derivatives: state-of-the-art, enhanced scenarios, and quality insights. Int J Mol Sci, 24:(10); 8465, 2023.

Venkatakrishna SS, Onyango LC, Serai SD, et al. "Kidney anatomy and physiology." In *Advanced Clinical MRI of the Kidney: Methods and Protocols*, pp. 3-12. Cham: Springer International Publishing, 2023.

Wen Q, Chen Q. An overview of ozone therapy for treating foot ulcers in patients with diabetes. Amer J Med Sci, 360:(2); 112-119, 2020.

Yang J, Wang X, Zeng X, et al. One-step stromal vascular fraction therapy in osteoarthritis with tropoelastin-enhanced autologous stromal vascular fraction gel. Front. Bioeng. Biotechnol., 12: 1359212, 2024.

Yang W, Li X, He L, et al. Empagliflozin improves renal ischemia–reperfusion injury by reducing inflammation and enhancing mitochondrial fusion through AMPK–OPA1 pathway promotion. Cellular & Molecular Biology Letters, 28:(1); 42, 2023.

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Exploring the therapeutic potential of honeybee venom in mitigating diabetic reproductive complications: insights from *in vitro* and *in vivo* studies in rats

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Abstract: Diabetes mellitus impairs male reproductive health through oxidative stress–mediated pathways. This study investigated the antioxidant capacity and reproductive effects of honeybee venom (apitoxin) in diabetic male rats. The *in vitro* antioxidant activity of bee venom showed a DPPH radical scavenging rate of 76.85 ± 1.55%, close to that of ascorbic acid (79.29 ± 1.82%, $P < 0.001$), and a metal chelating activity of 91.69 ± 2.55% ($P = 0.227$). For *in vivo* evaluation, streptozotocin-induced diabetic Wistar rats (n = 6/group) were assigned to control (C), apitoxin-treated (A), diabetic (D), and diabetic + apitoxin (DA) groups for 28 days. Diabetic rats exhibited markedly decreased sperm motility (61.4 ± 6.9%) compared to C (78.6 ± 3.8%) and A (82.9 ± 4.9%) ($P = 0.007$), whereas apitoxin treatment in DA rats improved motility to 77.1 ± 7.6%. Epididymal, vesicular, and prostatic weights were reduced in diabetic groups ($P < 0.01$). Oxidative stress was evident with elevated MDA in D (49.3 ± 31.1 nmol/mg protein) versus DA (27.7 ± 4.4) and C (29.0 ± 3.0) ($P = 0.023$), while GSH was significantly higher in DA (54.4 ± 18.4 µmol/mg protein) compared to D (35.2 ± 4.2) ($P = 0.006$). Overall, honeybee venom ameliorated diabetes-induced oxidative damage and improved sperm quality through redox modulation. These findings highlight its potential as a natural therapeutic agent against diabetic reproductive complications.
Keywords: Honeybee venom, diabetes, oxidative stress, sperm motility, antioxidant activity.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder that adversely affects quality of life in both humans and animals (Sharma et al., 2017). This detrimental effect primarily stems from the disruption of redox homeostasis within biological tissues, leading to an imbalance between oxidant generation and antioxidant defense (Korac et al., 2021). The excessive generation of reactive oxygen and nitrogen species (ROS and RNS) within tissues and organs disrupts metabolic pathways. It impairs cells sensitive to metabolic alterations, potentially leading to cellular and tissue demise. DM is characterized by significant disturbances in both glucose and fatty acid metabolism (Sivri, 2025). In managing DM, lifestyle modifications, dietary adjustments, and stress management strategies are recommended in conjunction with medications, including insulin tailored to the specific type of diabetes (Rosenfeld et al., 2025).

Given the established role of oxidative stress in diabetic complications, antioxidant-based interventions have gained interest, and bee venom presents a promising natural candidate due to its bioactive peptide components (Al-Hatamleh, 2020).
The male reproductive system is particularly vulnerable to diabetes-induced oxidative and metabolic alterations (Leisegang, 2022). Vascular, neural, and myopathic impairments contribute to reproductive dysfunction in males, thereby impacting sperm parameters as well. Perturbations in seminal activity parameters resulting from DM are often associated with infertility concerns (Gandhi et al., 2017). The susceptibility of spermatozoa membranes to peroxidative damage, owing to their lipid density, has been well-documented (Hwang, 2025), with previous reports outlining the effects of lipid peroxidation on semen quality in rats with experimental STZ-induced diabetes (Badejogbin, 2025).

Honeybee venom (apitoxin) from *Apis mellifera* is a complex mixture of peptides, enzymes, and bioactive molecules. Apitoxin has found therapeutic applications due to its recognized anti-inflammatory, antioxidant, and immune-modulating effects (Kasozi, 2020). Studies have indicated that administering apitoxin (0.1-0.3 mg per rabbit, subcutaneously) to male rabbits for 20 weeks enhances reproductive performance and antioxidant capacity (El-Hanoun et al., 2020). Similarly, research has shown that administering apitoxin at precise dosages, for specific durations, and via appropriate injection methods in female rats improves reproductive and immune performance (Elkomy et al., 2021). However, limited studies have examined the influence of bee venom on testicular redox balance and reproductive performance under diabetic conditions, particularly regarding semen quality, organ weights, and oxidative stress markers such as MDA, GSH, and NOx.

This study aimed to investigate the antioxidant capacity and reproductive effects of honeybee venom in diabetic male rats. Specifically, the objectives were:

1. To assess the *in vitro* antioxidant activity of honeybee venom using standard biochemical assays.
2. To evaluate the *in vivo* influence of honeybee venom administration on semen quality, testicular redox status, and reproductive organ morphology in streptozotocin-induced diabetic rats.
3. To examine the potential association between oxidative stress modulation and reproductive outcomes under diabetic conditions.

It was hypothesized that honeybee venom, owing to its bioactive constituents such as melittin and phospholipase A_2, may improve antioxidant status and testicular function by mitigating oxidative stress and supporting sperm quality in diabetic rats.

2. Materials e Methods

2.1. Animals and the experimental stage

In our investigation, a cohort of 40 male Wistar Albino rats, aged 3 months, procured from the Afyon Kocatepe University Experimental Animals Research and Application Center (Ref No: 49533702/97-445-15), were employed. All procedures were designed and conducted in accordance with the ARRIVE 2.0 guidelines (Du Sert et al., 2020), ensuring appropriate animal welfare, minimizing the number of animals used, and providing detailed reporting of experimental design, randomization, and blinding. Before the commencement of the study, all rats underwent a ten-day acclimatization period in polycarbonate cages, maintained under a controlled environment of 12-hour light/dark cycles, 65% relative humidity, and a temperature of 20 ± 1°C. Throughout the study duration, *ad libitum* access to standard laboratory food and water was provided.

Rats were randomly assigned to experimental groups, and the investigators conducting injections and data collection were blinded to group allocation. Diabetes mellitus was induced in 20 rats by intraperitoneal injection of streptozotocin (STZ, CAS No. 18883-66-4; Sigma-Aldrich, Darmstadt, Germany) at a dose of 50 mg/kg body weight, freshly dissolved in citrate buffer (pH 4.5) at room temperature immediately before injection, ten days before the commencement of the study, following the protocol outlined by Furman (2015). Rats were fasted for 12 hours before STZ administration, and 10% sucrose was added to their drinking water on the first day to prevent hypoglycemia. Normal tap water was restored on the second day. Diabetes induction was confirmed by measuring fasting blood glucose levels 72 hours after STZ injection using a glucometer, with a threshold of ≥200 mg/dl indicating diabetic status (Zhang 2025). No mortality was observed among diabetic rats following STZ injection.

Apitoxin is a complex mixture of bioactive compounds (Aufschnaiter et al., 2020). The three main components of honeybee venom from *Apis mellifera* are melittin, phospholipase A_2, and apamin. Molecular structures of these components were obtained from the Protein Data Bank (PDB IDs: melittin 2MW6; phospholipase A_2 1POC; apamin 7OXF) and visualized using PyMOL (version 2.5.5, Schrödinger, LLC) (Figure 1). High-purity apitoxin (Sigma-Aldrich, V3375) was freshly prepared daily in saline (154 mmol/l NaCl) at 0.5 mg/ml (w/v) and administered intraperitoneally at 0.5 mg/kg per rat, following the protocol of Mousavi et al. (2012). This dose was selected based on previous studies demonstrating safety and efficacy in rodents.

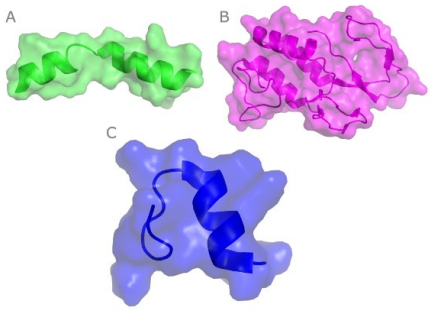

Figure 1 – Three main bioactive components of honeybee venom. The main three bioactive components of honeybee venom are presented in the figure. A depicts melittin, B shows phospholipase A_2, and C represents apamin. Peptide structures of these components were generated using PyMOL, employing the alpha-helix and beta-sheet methods, with the addition of a transparent outer layer using the molecular surface technique.

The experimental groups comprised 10 rats each: control (C), apitoxin-treated (A), diabetes-induced (D), and diabetes-induced apitoxin-treated (DA). The experiment lasted 28 days. Control and diabetes-induced groups received daily intraperitoneal saline injections, whereas apitoxin-treated groups received daily injections of freshly prepared apitoxin.

The *in vitro* antioxidant activity of apitoxin was assessed using DPPH radical scavenging activity (RSA) and metal chelating activity (MCA) assays. Apitoxin at 0.5 mg/ml and ascorbic acid, a standard antioxidant, were analyzed in triplicate, with three independent samples per assay. Samples were prepared in 200 µL volumes per well in a 96-well plate, and the reactions were incubated at room temperature for 30 minutes before measuring absorbance. The DPPH assay evaluates antioxidant activity by measuring the ability of antioxidants to neutralize 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals (Sigma-Aldrich), which are quantified spectrophotometrically at 520 nm (Munteanu and Apetrei, 2021; Pinto et al., 2021). The MCA assay assesses metal-chelating activity by measuring competition with 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p′-disulfonic acid monosodium salt hydrate (FerroZine®, Sigma-Aldrich) for Fe^{2+} ions. Chelation was quantified at 562 nm after a 30-minute incubation at room temperature (Dontha, 2016; Gulcin and Alwasel, 2022).

At the end of the experiment, rats were fasted overnight and anesthetized via intramuscular injection of Xylazine HCl (10 mg/kg) followed by Ketamine HCl (50 mg/kg). Rats were monitored for loss of reflexes and absence of pain response for 5 minutes to ensure adequate anesthesia. Euthanasia was performed by exsanguination under anesthesia, with continuous monitoring to confirm complete cessation of cardiac and respiratory activity.

2.2. Measurement of reproductive organ parameters

Following euthanasia, the testes, epididymides, seminal vesicles, and prostate glands were promptly extracted intact and rinsed with phosphate-buffered saline. Reproductive organ weights were measured using an analytical balance with 0.01 g precision.

Epididymal sperm samples were obtained from the cauda epididymis, and a small aliquot was placed on a preheated microscope slide at 37°C containing Tris buffer solution (20 mM), as previously described by Li et al. (2022).

Sperm motility was assessed under a phase-contrast microscope at 200x and 400x magnification, examining three different fields per sample to calculate average forward-progressive motility percentages. Abnormal spermatozoa were evaluated as percentages, based on standard morphological criteria (head, midpiece, and tail defects). To assess abnormal spermatozoa rates, samples were treated with Hancock solution and evaluated using the liquid fixation method detailed by Güngör et al. (2023). Specifically, at least three drops (~50 µL each) of sperm sample were combined with 1 ml of Hancock solution in Eppendorf tubes and gently mixed for 30 seconds. A drop of the mixture was transferred to a microscope slide, covered with a coverslip, and examined under oil immersion (1000x magnification, immersion oil with refractive index 1.515). Four hundred spermatozoa per field were counted, and abnormalities in the head, midpiece, and tail were recorded to calculate the abnormal spermatozoa percentage (Yeni, 2022).

2.3. Measurement of testicular redox parameters

To measure testicular redox parameters, approximately 0.5 g of testis tissue was homogenized in 0.05 M phosphate buffer (pH 7.0) using a glass-Teflon homogenizer at 10,000 rpm for 60 seconds and centrifuged at 3000×g for 15 minutes. The resulting supernatants were collected for subsequent assays.

Lipid peroxidation was determined as malondialdehyde (MDA) equivalents using the thiobarbituric acid reactive substances (TBARS) method (Ohkawa et al., 1979; Moradi, 2024). For this assay, 0.1 ml of supernatant was mixed with 0.2 ml of 8.1% sodium dodecyl sulfate and 1.5 ml of 0.8% thiobarbituric acid. Tubes were vortexed for 10 seconds, boiled in a 95°C water bath for 60 minutes, and cooled under running tap water for 5 minutes. Then, 5 ml of a n-butanol: pyridine (15:1) mixture and 1 ml of distilled water were added, and the samples were centrifuged at 2200×g for 10 minutes. The absorbance of the colored supernatant was measured spectrophotometrically at 532 nm using a Shimadzu UV-1201 spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

Reduced glutathione (GSH) levels were measured using previously described methods (Tietze, 1969; Keshta, 2023). Briefly, 0.2 ml of supernatant was mixed with 3 ml of ice-cold metaphosphoric acid solution (5 g metaphosphoric acid, 1 g EDTA, 90 g NaCl in 300 ml distilled water) and vortexed. After 5 minutes, the mixture was filtered using Whatman No. 1 filter paper (Cytiva, Maidstone, UK), and 2 ml of filtrate was combined with 8 ml of 0.05 M phosphate buffer and 0.5 ml of DTNB. Absorbance was measured at 412 nm against a blank containing 2 ml of distilled water and 3 ml of metaphosphoric acid solution using a UV–Vis spectrophotometer (Shimadzu UV-1201, Shimadzu Corporation, Kyoto, Japan).

Nitric oxide (NOx) levels were measured according to the Griess method (Cortas and Wakid, 1990; Saka, 2024). Proteins were removed using Somogyi's method (Sadeghi, 2022). For the assay, 50 µl of homogenate was mixed with 200 µl of Somogyi reagent (1 g NaOH and 5 g ZnSO4 in 50 ml distilled water each) and incubated overnight. Samples were centrifuged at 3000g for 15 minutes, then 100 µl of $VaCl_3$ solution (160 mg $VaCl_3$ dissolved in 20 ml 1 M HCl), 50 µl sulphanilamide, and 50 µl N-(1-Naphthyl) ethylenediamine were added. Mixtures were incubated at 40 °C for 30 minutes, and the absorbance was read at 545 nm against distilled water using a UV–Vis spectrophotometer (Shimadzu UV-1201, Shimadzu Corporation, Kyoto, Japan).

2.4. Statistical analysis

The results are presented as mean ± standard deviation (S.D.). Normality of data distribution was assessed for each parameter (e.g., AOA, reproductive organ weights, sperm motility, abnormal sperm rates, testicular MDA, GSH, NOx) using Shapiro-Wilk and Kolmogorov-Smirnov tests. For the *in vitro* antioxidant activity (AOA) comparisons, two-tailed unpaired t-tests were applied. When the assumption of homogeneity of variances was violated (as assessed by Levene's test), statistical comparisons were conducted using Welch's ANOVA, followed by post hoc Games-Howell tests to determine differences between groups. All analyses were performed using SPSS software (version 20, IBM Corp., Armonk, USA), with statistical significance set at $P < 0.05$.

3. Results

In vitro antioxidant activity (AOA) of bee venom was high. Bee venom exhibited significantly lower DPPH radical scavenging activity (76.85 ± 1.55%) compared to ascorbic acid (79.29 ± 1.82%, $P < 0.001$). In contrast, no significant difference was observed in metal chelating activity (MCA) between bee venom (91.69 ± 2.55%) and ascorbic acid (91.14 ± 2.57%, $P = 0.227$) (Table 1).

	DPPH	MCA
Bee venom	76.85 ± 1.55[a]	91.69 ± 2.55
Ascorbic acid	79.29 ± 1.82[b]	91.14 ± 2.57
p	< 0.001	0.227

Table 1 – Average RSA and MCA percentages of venom and ascorbic acid diluted in saline solution. The values presented in the table indicate the mean ± S.D. The letters "a" and "b" within the respective columns denote significant statistical disparities between groups ($P < 0.05$).

Reproductive organ measurements and sperm parameters are summarized in Table 2. Epididymis weights were significantly reduced in the diabetic (D, 0.45 ± 0.09 g) and diabetic + apitoxin (DA, 0.46 ± 0.08 g) groups compared to control (C, 0.64 ± 0.06 g) and apitoxin-treated (A, 0.59 ± 0.05 g) groups ($P = 0.003$). Vesicula seminalis and prostate weights showed significant decreases in group D (0.26 ± 0.09 g and 0.16 ± 0.04 g, respectively) and DA (0.38 ± 0.15 g and 0.23 ± 0.06 g) compared to control and A groups ($P = 0.002$ and $P = 0.005$, respectively).

Parameters	C	A	D	DA	P
Testis weight (g)	1.55 ± 0.12	1.45 ± 0.06	1.36 ± 0.16	1.45 ± 0.13	0.08
Epididymis weight (g)	0.64 ± 0.06[a]	0.59 ± 0.05[a]	0.45 ± 0.09[b]	0.46 ± 0.08[b]	0.003*
Vesicula seminalis weight (g)	0.84 ± 0.21[a]	0.67 ± 0.08[b]	0.26 ± 0.09[c]	0.38 ± 0.15[c]	0.002*
Prostate weight (g)	0.40 ± 0.11[a]	0.32 ± 0.03[b]	0.16 ± 0.04[c]	0.23 ± 0.06[c]	0.005*
Motility (%)	78.57 ± 3.77[a]	82.85 ± 4.87[a]	61.42 ± 6.90[b]	77.14 ± 7.55[a]	0.007*
Abnormal spermatozoon rate	12.35 ± 1.28[a]	7.78 ± 1.18[b]	12.92 ± 2.04[a]	11.50 ± 0.95[a]	0.009*

Table 2 – Means of reproductive organ parameters. Significant statistical variances were observed among groups, as denoted (data presented as mean ± S.D., n = 6; determined via Welch ANOVA, posthoc Games-Howell test, *P < 0.05; a,b,c: distinct letters on the same line signify intergroup disparities). Abbreviations used: C for control, A for apitoxin-treated, D for diabetic, and DA for apitoxin-treated diabetic groups.

Sperm motility was significantly lower in the diabetic group (D, 61.42 ± 6.90%) compared to control (C, 78.57 ± 3.77%), A (82.85 ± 4.87%), and DA (77.14 ± 7.55%) groups (P = 0.007). The lowest abnormal spermatozoon rate was observed in the apitoxin-treated control group (A, 7.78 ± 1.18%), significantly lower than control (C, 12.35 ± 1.28%) and diabetic (D, 12.92 ± 2.04%) groups (P = 0.009).

Analysis of testicular redox parameters revealed significant differences among groups (Figure 2). MDA levels were highest in group D (49.30 ± 31.06 nmol/mg protein), significantly higher than in the DA (27.72 ± 4.39) and C (28.98 ± 3) groups (P = 0.023). GSH levels peaked in group DA (54.4 ± 18.4 µmol/mg protein), which was significantly higher than group D (35.24 ± 4.21) and statistically similar to control (44.06 ± 9.25) and A (40.57 ± 8.43) groups (P = 0.006). NOx levels were significantly elevated in the apitoxin-treated control group (A, 8.33 ± 1.49 µmol/mg protein) compared to all other groups, while the diabetic group (D, 1.51 ± 0.25) had the lowest levels (P < 0.001).

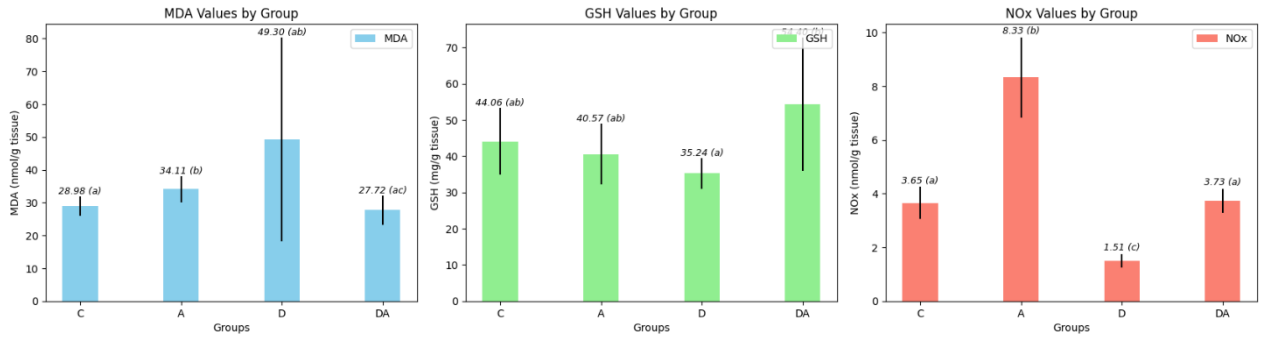

Figure 2 – Testicular tissue redox parameters. Bar graphs display the quantification, represented with various units and ±S.D., of malondialdehyde (MDA), reduced glutathione (GSH), and nitric oxide (NOx) parameters assessed among study groups (statistical analysis conducted using Welch ANOVA, posthoc Games-Howell test, P < 0.05). Group abbreviations: C for control, A for apitoxin-treated, D for diabetic, and DA for apitoxin-treated diabetic groups. Variances between groups are indicated by distinct letters.

4. Discussion

The results of this study indicate that bee venom exhibits considerable *in vitro* antioxidant activity. When assessed using DPPH radical scavenging activity, bee venom showed slightly lower activity compared to ascorbic acid (P < 0.001), whereas no significant difference was observed in metal chelating activity (P = 0.227). Similar trends have been reported in *Apis mellifera syriaca* venom, showing dose-dependent DPPH activity with slightly lower radical-scavenging activity than ascorbic acid. In contrast, metal chelation activity remained high, highlighting the potential of venom peptides in mitigating metal-induced oxidative damage (Frangieh et al., 2019). As observed in our previous study (Denk, 2023), the validity of optimal AOA for bee venom dissolved in saline solution has been confirmed in this study.

In terms of testes weights, no significant differences were observed among groups. This aligns with previous studies in which diabetic conditions did not significantly alter testicular weight, whereas accessory sex organs such as the epididymides, seminal vesicles, and prostate glands exhibited reductions (Atta et al., 2017; Soudamani et al., 2005; Zhao et al., 2021). Notably, apitoxin administration in healthy animals also led to decreased weights of seminal vesicles and prostate, which may be attributed to potential redox modulation or mild pro-oxidant effects of apitoxin (Denk and Fidan, 2021). These findings indicate that changes in organ weights may result not only from diabetic oxidative stress but also from apitoxin dosage and treatment duration.

Sperm motility was significantly reduced in diabetic rats, consistent with previous observations linking hyperglycemia-induced oxidative stress and lipid peroxidation to impaired sperm function (Khoei et al., 2019; Zhao et al., 2021; Zhu et al., 2021). Interestingly, apitoxin treatment partially restored motility in diabetic rats, suggesting a modulatory effect on the *in vivo* antioxidant capacity of the testes and epididymides, improving sperm function.

Regarding abnormal spermatozoa, the rate increased in diabetic rats as expected, reflecting oxidative damage to sperm membranes and DNA (Minas, 2024). Apitoxin treatment in healthy rats reduced the incidence of abnormal spermatozoa, consistent with previous reports highlighting its protective and antioxidant effects in reproductive tissues (El-Hanoun et al., 2020). Differences in species, dosage, and administration route may explain discrepancies between studies.

Testicular redox parameters were affected differently across groups. MDA levels were elevated in diabetic rats, while GSH levels were decreased, reflecting oxidative and nitrosative stress associated with diabetes (Szabo, 2009; Tousoulis et al., 2012). Apitoxin administration in diabetic rats increased GSH and normalized NOx levels, consistent with reported antioxidant and redox-modulatory properties of melittin and phospholipase A_2 (Yoon et al., 2008). However, in healthy rats, apitoxin increased MDA and NOx levels without significantly affecting GSH, suggesting organ-specific and dose-dependent responses.

Overall, the study demonstrates that bee venom possesses notable *in vitro* antioxidant capacity and modulatory effects on reproductive parameters *in vivo*. These effects are consistent with previous studies indicating that apitoxin peptides can regulate intracellular oxidative stress and modulate redox balance (Denk, 2021) and can improve sperm function (Suleiman, 2021; Abdelhamid, 2023), although the magnitude of these responses may vary with metabolic status and dosage. While *in vitro* assays provide insight, *in vivo* outcomes highlight complex interactions between bee venom components, diabetic oxidative stress, and reproductive function.

5. Conclusion

This study demonstrates that honey bee venom exhibits notable antioxidant activity and modulates oxidative stress and reproductive parameters in diabetic rats. Although its *in vitro* radical scavenging capacity was slightly lower than that of ascorbic acid, bee venom maintained comparable metal chelating activity. It also exhibited the ability to modulate redox balance *in vivo*. The observed increase in reduced glutathione and normalization of nitric oxide levels in apitoxin-treated diabetic rats indicate its potential to mitigate oxidative and nitrosative stress. Moreover, improvements in sperm motility and reductions in abnormal spermatozoa rates suggest a protective influence of apitoxin on reproductive function under diabetic conditions. Collectively, these findings highlight the capacity of bee venom to influence redox homeostasis and reproductive health, supporting its possible role as a bioactive modulator within the context of diabetes-induced oxidative damage.

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6. References

Abdelhamid MS, El Bohi KM, Sherif MH, et al. Bee venom ameliorates methyl mercury-induced reproductive impairment in male Sprague Dawley rats. Biochemistry Lett, 19:(1);138–150, 2023. (DOI: https://doi.org/10.21608/blj.2025.231715.1045)

Al-Hatamleh MA, Boer JC, Wilson KL, et al. Antioxidant-based medicinal properties of stingless bee products: Recent progress and future directions. Biomolecules, 10:(6);923, 2020. (DOI: https://doi.org/10.3390/biom10060923)

Atta MS, Almadaly EA, El-Far AH, et al. Thymoquinone defeats diabetes-induced testicular damage in rats targeting antioxidant, inflammatory and aromatase expression. Int J Mol Sci, 18:(5);919, 2017. (DOI: https://doi.org/10.3390/ijms18050919)

Aufschnaiter A, Kohler V, Khalifa S, et al. Apitoxin and its components against cancer, neurodegeneration and rheumatoid arthritis: Limitations and possibilities. Toxins, 12:(2);66, 2020. (DOI: https://doi.org/10.3390/toxins12020066)

Badejogbin OC, Chijioke-Agu OE, Olubiyi MV, et al. Pathogenesis of testicular dysfunction in diabetes: exploring the mechanism and therapeutic interventions. J Assist Reprod Genet, 42:(2);367–379, 2025. (DOI: https://doi.org/10.1007/s10815-024-03314-3)

Cortas NK, Wakid NW. Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method. Clin Chem, 36:(8);1440–1443, 1990. (DOI: https://doi.org/10.1093/clinchem/36.8.1440)

Denk B, Fidan AF, et al. Effects of honeybee (Apis mellifera) venom on redox balance, biochemical and hematological profile in diabetic rats: A preliminary study. Türk J Vet Anim Sci, 45:(2);257–265, 2021. (DOI: https://doi.org/10.3906/vet-2006-139)

Denk B. Exploring Apis mellifera L. Venom's Antioxidant Power in Various Solvents: Unveiling its *In vitro* Potential. Kocatepe Vet J, 16:(3);420–431, 2023. (DOI: https://doi.org/10.30607/kvj.1343130)

Dontha S. A review on antioxidant methods. Asian J Pharm Clin Res, 9:(2);14–32, 2016. (DOI: http://dx.doi.org/10.22159/ajpcr.2016.v9s2.13092)

Du Sert NP, Ahluwalia A, Alam S, et al. Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. PLoS biology, 18:(7);e3000411, 2020. (DOI: https://doi.org/10.1371/journal.pbio.3000411)

El-Hanoun A, El-Komy A, El-Sabrout K, et al. Effect of bee venom on reproductive performance and immune response of male rabbits. Physiol Behav, 223:(1);112987, 2020. (DOI: https://doi.org/10.1016/j.physbeh.2020.112987)

Elkomy A, El-Hanoun A, Abdella M, et al. Improving the reproductive, immunity and health status of rabbit does using honey bee venom. J Anim Physiol Anim Nutr, 105:(5);975–983, 2021. (DOI: https://doi.org/10.1111/jpn.13552)

Frangieh J, Salma Y, Haddad K, et al. First characterization of the venom from Apis mellifera syriaca, a honeybee from the Middle East region. Toxins, 11:(4);191, 2019. (DOI: https://doi.org/10.3390/toxins11040191)

Furman BL. Streptozotocin-induced diabetic models in mice and rats. Curr Protoc Pharmacol, 70:(1);5–47, 2015. (DOI: https://doi.org/10.1002/0471141755.ph0547s70)

Gandhi J, Dagur G, Warren K, et al. The role of diabetes mellitus in sexual and reproductive health: an overview of pathogenesis, evaluation, and management. Curr Diabetes Rev, 13:(6);573–581, 2017. (DOI: https://doi.org/10.2174/1573399813666161122124017)

Gulcin İ, Alwasel SH. Metal ions, metal chelators and metal chelating assay as antioxidant method. Processes, 10:(1);132, 2022. (DOI: https://doi.org/10.3390/pr10010132)

Güngör Ş, Yeni D, İnanç M, et al. Evaluation of the *in vitro* cryopreservative performance of Juniper berry oil (Juniperus communis) on frozen-thawed bull semen. Acta Vet Brno, 92:(4);335-342, 2023. (DOI: http://doi.org/10.2754/avb202392040335)

Hwang YY, Tsen QT, Felim J, et al. Probiotics as a therapeutic approach to alleviate reproductive harm from polystyrene microplastics in male rats. Sci Rep, 15:(1);34783, 2025. (DOI: https://doi.org/10.1038/s41598-025-18550-5)

Kasozi KI, Niedbała G, Alqarni M, et al. Bee venom—a potential complementary medicine candidate for SARS-CoV-2 infections. Front Public Health, 8:(1);594458, 2020. (DOI: https://doi.org/10.3389/fpubh.2020.594458)

Keshta AT, Fathallah AM, Attia YA, et al. Ameliorative effect of selenium nanoparticles on testicular toxicity induced by cisplatin in adult male rats. Food Chem Toxicol, 179:(1);113979, 2023. (DOI: https://doi.org/10.1016/j.fct.2023.113979)

Khoei HH, Fakhri S, Parvardeh S, et al. Testicular toxicity and reproductive performance of streptozotocin-induced diabetic male rats: the ameliorating role of silymarin as an antioxidant. Tox Rev, 38:(3);223-233, 2019. (DOI: https://doi.org/10.1080/15569543.2018.1444641)

Korac B, Kalezic A, Pekovic-Vaughan V, et al. Redox changes in obesity, metabolic syndrome, and diabetes. Redox Biol. 42; 101887, 2021. (DOI: https://doi.org/10.1016/j.redox.2021.101887)

Leisegang K, et al. Oxidative stress in men with obesity, metabolic syndrome and type 2 diabetes mellitus: Mechanisms and management of reproductive dysfunction. In: Oxidative Stress and Toxicity in Reproductive Biology and Medicine: A Comprehensive Update on Male Infertility-Volume One. Cham: Springer International Publishing, 237–256, 2022. (DOI: https://doi.org/10.1007/978-3-030-89340-8_11)

Li H, Huo Y, He X, et al. A male germ-cell-specific ribosome controls male fertility. Nature, 612:(7941);725–731, 2022. (DOI: https://doi.org/10.1038/s41586-022-05508-0)

Minas A, Camargo M, Alves MG, et al. Effects of diabetes-induced hyperglycemia on epigenetic modifications and DNA packaging and methylation during spermatogenesis; A narrative review. Iran J Basic Med Sci, 27:(1);3, 2024. (DOI: https://doi.org/10.22038/IJBMS.2023.69604.15173)

Moradi M, Jahromi MG, Mirzaei S, et al. Protective Effects of Scrophularia striata Extract on Testicular Function and Spermatogenesis in Cadmium-Exposed Mice: Role of Nitro-Oxidative Stress Modulation. Andrologia, 2024:(1);5565361, 2024. (DOI: https://doi.org/10.1155/and/5565361)

Mousavi SM, Imani S, Haghighi S, et al. Effect of Iranian honey bee (Apis mellifera) venom on blood glucose and insulin in diabetic rats. J Arthropod-Borne Dis, 6:(2);136, 2012.

Munteanu IG, Apetrei C. Analytical methods used in determining antioxidant activity: A review. Int J Mol Sci, 22:(7);3380, 2021. (DOI: https://doi.org/10.3390/ijms22073380)

Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem, 95:(2);351–358, 1979. (DOI: https://doi.org/10.1016/0003-2697(79)90738-3)

Pinto D, Vieira EF, Peixoto AF, et al. Optimizing the extraction of phenolic antioxidants from chestnut shells by subcritical water extraction using response surface methodology. Food Chem. 334; 127521, 2021. (DOI: https://doi.org/10.1016/j.foodchem.2020.127521)

Rosenfeld RM, Grega ML, Karlsen MC, et al. Lifestyle interventions for treatment and remission of type 2 diabetes and prediabetes in adults: a clinical practice guideline from the American College of Lifestyle Medicine. Am J Lifestyle Med, 19:(2_suppl);10S–131S, 2025. (DOI: https://doi.org/10.1177/15598276251325488)

Sadeghi R, Fang F, Shao Y, et al. Eliminating protein interference when quantifying potato reducing sugars with the miniaturized Somogyi-Nelson assay. Food Chem, 373:(1);131473, 2022. (DOI: https://doi.org/10.1016/j.foodchem.2021.131473)

Saka WA, Adeogun AE, Adisa VI, et al. L-arginine attenuates dichlorvos-induced testicular toxicity in male Wistar rats by suppressing oxidative stress-dependent activation of caspase 3-mediated apoptosis. Biomed Pharmacother, 178:(1);117136, 2024. (DOI: https://doi.org/10.1016/j.biopha.2024.117136)

Sharma S, Mathew AB, Chugh J. miRNAs: Nanomachines that micromanage the pathophysiology of diabetes mellitus. Adv Clin Chem, 82;199–264, 2017. (DOI: https://doi.org/10.1016/bs.acc.2017.06.003)

Sivri D, Akdevelioğlu Y. Effect of fatty acids on glucose metabolism and type 2 diabetes. Nutr Rev, 83:(5);897–907, 2025. (DOI: https://doi.org/10.1093/nutrit/nuae165)

Soudamani S, Yuvaraj S, Malini T, et al. Experimental diabetes has adverse effects on the differentiation of

ventral prostate during sexual maturation of rats. Anat Rec A, 287:(2);1281–1289, 2005. (DOI: https://doi.org/10.1002/ar.a.20250)

Suleiman JB, Bakar ABA, Mohamed M. Review on bee products as potential protective and therapeutic agents in male reproductive impairment. Molecules, 26:(11);3421, 2021. (DOI: https://doi.org/10.3390/molecules26113421)

Szabo C. Role of nitrosative stress in the pathogenesis of diabetic vascular dysfunction. Br J Pharmacol, 156:(5);713–727, 2009. (DOI: https://doi.org/10.1111/j.1476-5381.2008.00086.x)

Tietze F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. Anal Biochem, 27:(3);502–522, 1969. (DOI: https://doi.org/10.1016/0003-2697(69)90064-5)

Tousoulis D, Kampoli A-M, Tentolouris N, et al. The role of nitric oxide on endothelial function. Curr Vasc Pharmacol, 10:(1);4–18, 2012. (DOI: https://doi.org/10.2174/157016112798829760)

Yeni D, Güngör Ş, Avdatek F, et al. Investigation of changes in spermatozoon characteristics, chromatin structure, and antioxidant/oxidant parameters after freeze-thawing of hesperidin (vitamin P) doses added to ram semen. Life, 12:(11);1780, 2022. (DOI: https://doi.org/10.3390/life12111780)

Yoon S-Y, Kwon Y-B, Kim H-W, et al. Bee venom injection produces a peripheral anti-inflammatory effect by activation of a nitric oxide-dependent spinocoeruleus pathway. Neurosci Lett, 430:(2);163–168, 2008. (DOI: https://doi.org/10.1016/j.neulet.2007.10.035)

Zhang A, Zhichen B, Kidoguchi S, et al. An SGLT2 inhibitor, canagliflozin, reduces blood glucose level in the renal capillaries and protects the capillary network in the diabetic rats. Diabetes Obes Metab, Early view;1–9, 2025. (DOI: https://doi.org/10.1111/dom.70118)

Zhao L, Makinde EA, Olatunji OJ, et al. Protective effects of ethyl acetate extract from Shorea roxburghii against diabetes induced testicular damage in rats. Environ Toxicol, 36:(3);374–385, 2021. (DOI: https://doi.org/10.1002/tox.23043)

Zhu Y, Du Q, Jiao N, et al. Catalpol ameliorates diabetes-induced testicular injury and modulates gut microbiota. Life Sci, 267;118881, 2021. (DOI: https://doi.org/10.1016/j.lfs.2020.118881)

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Integrative effects of long-acting progesterone and micronutrient-amino acid complex on pregnancy maintenance in Bos indicus females under field conditions

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Abstract: The present study aimed to evaluate the pregnancy rate following fixed-time artificial insemination (FTAI) in Nelore cattle supplemented with either long-acting progesterone (Experiment 1) or an injectable nutritional complex (Experiment 2). In Experiment 1, multiparous cows underwent estrus synchronization and were inseminated on day D10 (D0 = first day of synchronization). On D15, animals were assigned to a control group (n=166), which received an intramuscular injection of 0.5 mL of 0.9% NaCl solution, or to a treated group (n=147), which received 0.5 mL of long-acting progesterone (150 mg). In Experiment 2, heifers were divided into a control group (n=108), which received no treatment, and a treated group (n=99), which received an intramuscular injection of a nutritional supplement on D0 of synchronization. After 7 days of FTAI, all cows and heifers were maintained with bulls for natural mating (20 females per bull) for the following 45 days. Pregnancy diagnosis was performed 70 days after FTAI. The pregnancy rate was higher in the treated groups for both experiments (53.1% vs. 42.8% in Experiment 1, and 53.7% vs. 38.8% in Experiment 2), although differences were not statistically significant ($p > 0.05$). In animals that did not conceive, ovarian cyclicity was not suppressed, indicating that the use of long-acting progesterone or nutritional supplementation did not interfere with the return to estrus. In conclusion, supplementation with long-acting progesterone or an injectable nutritional complex tended to improve pregnancy rates in Nelore cattle subjected to FTAI, without residual inhibitory effects on subsequent ovarian activity.
Keywords: cow, estrus-synchronization, heifer, FTAI, Nelore.

1. Introduction

Nutritional status is a critical determinant of reproductive efficiency in beef cattle, influencing follicular development, luteal function, oocyte competence, and early embryonic survival. In tropical production systems, cows frequently experience mineral and vitamin deficiencies due to the seasonal variation in forage quality and high metabolic demands during lactation. These nutritional imbalances impair ovarian function and endocrine signaling, leading to suboptimal responses to synchronization and fixed-time artificial insemination (FTAI) protocols (Diskin & Kenny, 2014; Rizzo et al., 2019).

Beyond the correction of macronutrient deficits, targeted supplementation with nutraceuticals—bioactive compounds such as trace minerals, vitamins, and amino acids—has emerged as a promising strategy to enhance reproductive outcomes. Micronutrients including zinc, copper, manganese, and selenium act as essential cofactors for antioxidant enzymes such as superoxide dismutase and glutathione peroxidase, protecting follicular cells from oxidative stress and improving steroidogenic activity (Arechiga et al., 1998; Bian et al., 2007). Vitamins A and E, along with β-carotene, contribute to follicular vascularization, luteal function, and conceptus development through retinoid and antioxidant mechanisms (Volker & Ludwig, 1995; Boxmeer et al., 2009). In mares, targeted nutritional supplementation with these compounds has been associated with improved follicular dynamics and higher embryo recovery rates, suggesting conserved physiological mechanisms across species (Aurich et al., 2018, 2019; Del Prete et al., 2024).

Injectable formulations of minerals and amino acids offer a rapid and bioavailable route to correct subclinical deficiencies, bypassing limitations of oral intake, ruminal degradation, and variable absorption. The metabolic support provided by such supplements may directly influence folliculogenesis and oocyte maturation, as well as modulate uterine receptivity through improved systemic oxidative balance. However, despite extensive studies on dietary supplementation, the reproductive effects of injectable nutraceutical complexes under field FTAI conditions remain poorly characterized.

Furthermore, hormonal modulation through exogenous progesterone administration represents another approach to optimize the endocrine environment during the peri-ovulatory period. Long-acting progesterone formulations may enhance luteal support and stabilize cyclicity in animals failing to conceive after FTAI, potentially sustaining ovarian activity and minimizing the anestrus period (Torres et al., 2021; Bó et al., 2023). When combined with targeted nutraceutical interventions, such strategies may synergistically improve follicular quality and pregnancy outcomes.

Therefore, this study was designed to test the hypothesis that injectable nutraceutical supplementation (trace minerals and amino acids) and long-acting progesterone administration can enhance reproductive performance in beef cows submitted to FTAI by improving ovarian function, luteal activity, and systemic metabolic balance.

The specific objectives of this study were: (1) to evaluate whether the administration of long-acting progesterone five days after FTAI influences cyclicity and pregnancy rate in multiparous cows, and (2) to determine the effect of injectable nutraceutical supplementation initiated at the onset of synchronization on follicular dynamics and conception rate in heifers.

2. Material and methods

2.1. Animals and management

Experiment 1 was carried out in the municipality of Porto Acre, Brazil (latitude −9.588° S, longitude −67.448° W), located in an equatorial climate with abundant rainfall. A total of 313 multiparous Nelore cows (Bos indicus) were selected, with a mean body weight of 430.0 ± 12.9 kg and an average age of 6.0 ± 0.6 years. Body condition score (BCS) averaged 3.0 ± 0.2 on a 1-to-5 scale (1 = emaciated; 5 = obese) according to Moraes et al. (2013). Animals grazed on Brachiaria brizantha mixed with MG-5 Vitória pasture and had ad libitum access to water and a commercial mineral supplement (PhósPrime®, Arantes Nutrição Animal, Ji-Paraná, Rondônia, Brazil) throughout the experimental period.

2.2. Estrus synchronization and progesterone treatment

Cows were enrolled on a random day of the estrous cycle, approximately 90 days postpartum, and synchronized according to the protocol illustrated in Figure 1.

On Day 0 (D0), each cow received an intravaginal progesterone device (1 g progesterone; Sincrogest®, Ouro Fino, Cravinhos, São Paulo, Brazil) and an intramuscular (IM) injection of 2 mL estradiol benzoate (2 mg; Sincrodiol®, Ouro Fino). On D8, the device was removed and the cows were treated IM with 1 mL estradiol cypionate (1 mg; SincroCP®, Ouro Fino), 1.5 mL eCG (300 IU; Sincro eCG®, Ouro Fino), and 2 mL sodium cloprostenol (0.530 mg; Sincrocio®, Ouro Fino). Fixed-time artificial insemination (FTAI) was performed on D10.

Five days later (D15), cows were randomly allocated into two groups:

- Control group (n = 166): received 0.5 mL of 0.9% NaCl IM.
- Treated group (n = 147): received 0.5 mL of long-acting progesterone (300 mg/mL; P4-300®, Botupharma, São Paulo, Brazil) IM.

To evaluate potential long-term effects of exogenous progesterone, all cows were maintained with fertile Nelore bulls (ratio = 1 bull per 20 cows) for 45 days following FTAI. Bulls (~5 years old) met breeding-soundness standards recommended by the Brazilian College of Animal Reproduction (CBRA, 2013): subjective motility ≥ 60%, minor defects ≤ 20%, major defects ≤ 10%, and ≥ 70% morphologically normal spermatozoa.

2.3. Experiment 2 – Effect of injectable nutraceutical supplementation on FTAI pregnancy rate

2.3.1. Animals and management

Experiment 2 was conducted in a herd located at latitude 11° 46′ 10″ S and longitude 61° 42′ 77″ W, also under an equatorial climate with abundant rainfall. A total of 207 Nelore heifers (Bos indicus) were enrolled, with an average body weight of 311.0 ± 48.1 kg and a mean BCS of 3.0 ± 0.5 (1–5 scale; Moraes et al., 2013). Heifers grazed on Brachiaria brizantha pastures and had unrestricted access to water and a mineral supplement (PhósPrime®, Arantes Nutrição Animal, Ji-Paraná, Brazil). Animals were randomly assigned to two groups:

- Control group (n = 108): received 5 mL of 0.9% NaCl IM.
- Treated group (n = 99): received 5 mL IM of a nutraceutical supplement containing amino acids, vitamins, and minerals. The supplement provides vitamins A, D3, E, B1, B5, B6, nicotinic and pantothenic acids, and multiple trace elements (Zn, Mn, Cu, Se, I, Fe, Co, Mg, Ca, P) together with amino acids (methionine, arginine, lysine, glycine, tryptophan) and inositol, with a recommended re-application interval of 3–4 months (Figure 1).

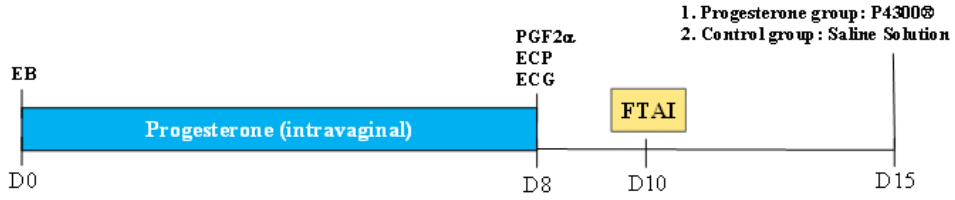

Figure 1 – Figure 1. Experimental design of Experiment 1 evaluating the effect of long-acting progesterone administration after fixed-time artificial insemination (FTAI) in Bos indicus cows. Legend: On Day 0 (D0), cows received estradiol benzoate (EB, 2 mg, IM) and an intravaginal progesterone device (1 g P_4), maintained until Day 8 (D8). On D8, the devices were removed and animals were treated intramuscularly with $PGF_{2}\alpha$ (0.530 mg), estradiol cypionate (1 mg), and equine chorionic gonadotrophin (300 IU). Fixed-time artificial insemination was performed on Day 10 (D10). On Day 15 (D15), cows were allocated into two groups: (1) Progesterone-treated group, receiving long-acting natural progesterone (300 mg; P4-300®); or (2) Control group, receiving physiological saline solution (0.9% NaCl).

2.3.2. Estrus synchronization and nutraceutical administration

The synchronization protocol began on a random day of the estrous cycle (Figure 2). On D0, all heifers received an intravaginal progesterone device (1 g; Sincrogest®, Ouro Fino) and 2 mL estradiol benzoate IM (2 mg; Sincrodiol®, Ouro Fino). At the same time, treated heifers received 10 mL IM of the nutraceutical supplement.

On D7, all heifers received 25 mg $PGF_{2}\alpha$ (dinoprost tromethamine; Lutalyse®, Zoetis, Guarulhos, Brazil). On D9, the devices were removed and animals were administered 0.3 mL estradiol cypionate (ECP®, Zoetis) and 300 IU eCG (Novormon®, Zoetis). FTAI was performed on D11. Seven days after FTAI, all heifers were exposed to fertile Nelore bulls (1 bull per 20 heifers) for 45 days to allow natural breeding. Bulls were ~5 years old and had been evaluated for breeding soundness according to CBRA (2013) guidelines.

2.4. Pregnancy diagnosis and evaluation of cyclicity

In both experiments, pregnancy diagnosis was performed 70 days after FTAI (D80) using transrectal ultrasonography with a 5 MHz linear probe (SD-20, Mindray do Brasil, São Paulo, Brazil; Figure 2).

Gestational age was used to differentiate pregnancies resulting from FTAI versus natural mating.

Additionally, the reproductive status of non-pregnant females was evaluated indirectly during the 45-day natural mating period following FTAI. The re-establishment of normal estrous cyclicity was inferred from comparable post-mating pregnancy rates between treated and control groups, indicating that neither long-acting progesterone nor injectable nutraceutical supplementation impaired ovarian function or estrous resumption.

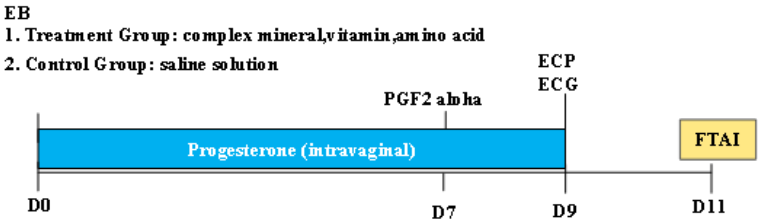

Figure 2 – Figure 2. Experimental design of Experiment 2 evaluating the effect of injectable mineral–vitamin–amino acid supplementation on the pregnancy rate after fixed-time artificial insemination (FTAI) in Bos indicus heifers. Legend: On Day 0 (D0), heifers received estradiol benzoate (2 mg, IM) and an intravaginal progesterone device (1 g P_4), maintained until Day 9 (D9). At the same time, animals in the treated group received an injectable mineral–vitamin–amino acid complex containing vitamins A (1.176 g), D_3 (0.025 g), E (0.5 g), B_1 (0.09 g), B_5 (1.0 g), and B_6 (0.09 g); nicotinic acid (0.65 g); pantothenic acid (1.0 g); trace minerals—selenium (0.06 g), zinc (0.15 g), manganese (0.1 g), copper (0.1 g), cobalt (0.1 g), iodine (0.2 g), magnesium (0.5 g), calcium (0.8 g), phosphorus (0.2 g), and iron (0.35 g); and amino acids—methionine (0.25 g), arginine (0.03 g), lysine (0.03 g), glycine (0.2 g), and tryptophan (0.06 g). On Day 7 (D7), all heifers were treated with $PGF_{2}\alpha$ (25 mg). On Day 9 (D9), after device removal, animals received estradiol cypionate (0.3 mg) and equine chorionic gonadotrophin (300 IU). Fixed-time artificial insemination was performed on Day 11 (D11).

2.5. Pregnancy diagnosis

In both Experiments, the pregnancy diagnosis was performed 70 days after the FTAI (D80) by ultrasonography, using a 5 MHz probe (SD.20, Mindray do Brasil, São Paulo, Brazil). The gestational age of each pregnancy was used to determine the pregnancy rate from FTAI and the natural service. In addition to pregnancy diagnosis, the reproductive status of non-pregnant females was indirectly assessed through their fertility response during the 45-day natural service period following FTAI. The re-establishment of normal estrous cyclicity was inferred from the comparable pregnancy rates between treated and control groups after natural mating, indicating that neither long-acting progesterone nor the micronutrient-amino acid complex interfered with ovarian function or estrous resumption.

2.6. Statistical analysis

Data were analyzed using the Statistical Analysis System (SAS®, version 9.4; SAS Institute Inc., Cary, NC, USA). Descriptive statistics were used to summarize animal characteristics (mean ± standard deviation for continuous variables and percentages for categorical variables). Pregnancy rate after FTAI and cumulative pregnancy rate after natural mating were analyzed as binary outcomes (pregnant = 1; non-pregnant = 0) using logistic regression models (PROC LOGISTIC). The models included treatment group (control or treated) as a fixed effect, while body condition score (BCS) and days postpartum at the start of synchronization were included as covariates to account for individual variability. The odds ratio (OR) and 95% confidence interval (CI) were estimated to quantify the strength of association between treatments and pregnancy outcomes.

Continuous variables such as body weight and BCS were compared between groups using Student's t-test, after confirming data normality with the Shapiro–Wilk test. When assumptions of normality were not met, the Wilcoxon rank-sum test was applied. Statistical significance was declared at $P < 0.05$, and tendencies were discussed when $0.05 \leq P \leq 0.10$. Results are presented as least squares means ± standard error of the mean (SEM) unless otherwise specified. To assess the potential influence of long-acting progesterone and nutraceutical supplementation on cyclicity resumption, a comparative analysis of post-FTAI natural-service pregnancy rates was performed using Pearson's chi-square test, evaluating whether treatment influenced the re-establishment of normal estrous activity among non-pregnant females.

3. Results

In Experiment 1, treatment with long-acting progesterone five days after FTAI did not affect pregnancy rate ($P > 0.05$). The proportion of pregnant cows was 53.1% (78/147) in the treated group and 42.8% (71/166) in the control group (Figure 3A). Similarly, in Experiment 2, no significant difference was observed between heifers supplemented with the injectable mineral–vitamin–amino acid complex (53.7%; 36/67) and control heifers (38.8%; 26/67) ($P > 0.05$; Figure 3B).

Bootstrap violin plots were used to illustrate the uncertainty distribution of pregnancy rates across treatment groups. Dots represent the observed pregnancy proportions, vertical bars indicate 95% Wilson confidence intervals, and violin shapes display the bootstrap density (20,000 resamples) for visual comparison between treated and control animals.

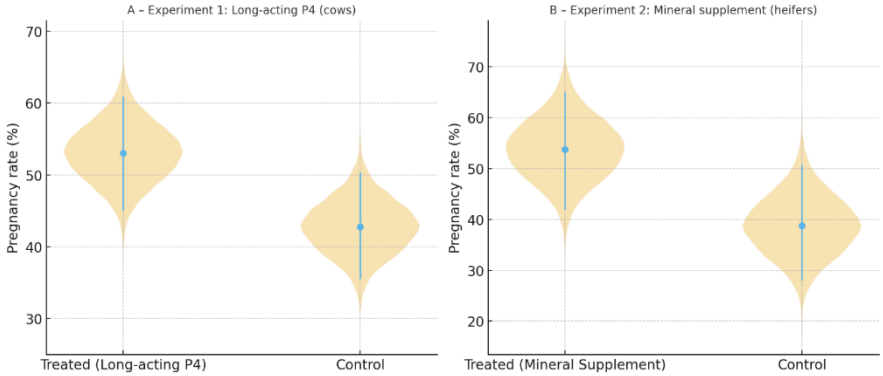

Figure 3 – Figure 3A. Bootstrap violin plots of pregnancy rates (FTAI) in Bos indicus cows and heifers. Panel A shows Bos indicus cows treated with long-acting injectable progesterone (P4) and controls (Experiment 1). Panel B shows Bos indicus heifers supplemented with a micronutrient and amino acid complex and controls (Experiment 2). Dots indicate observed pregnancy rates (%), and vertical bars represent 95% Wilson confidence intervals. Violins display the bootstrap distribution (20,000 resamples) of pregnancy outcomes, illustrating uncertainty around group proportions.

After the natural mating period, neither treatment significantly influenced the subsequent pregnancy rate among females that had failed to conceive after FTAI ($P > 0.05$; Table 1). In Experiment 1, pregnancy rates after natural service were 58.9% (56/95) for treated cows and 49.3% (34/69) for controls (Absolute Risk Difference = +9.7 pp; 95% CI: −5.7 to +25.1; OR = 1.48; one-sided Fisher's $P = 0.142$).

In Experiment 2, the corresponding rates were 75.6% (31/41) in supplemented heifers and 58.0% (18/31) in controls (Absolute Risk Difference = +17.6 pp; 95% CI: −4.2 to +39.3; OR = 2.24; one-sided Fisher's $P = 0.093$).

Supplementation type	Pregnancy rate Treated N (%)	Pregnancy rate Control N (%)	Absolute Risk Difference (95% CI)	Odds Ratio (95% CI)	Fisher *P*
Long-acting progesterone (P4-300)	56/95 (58.9)	34/69 (49.3)	+9.7 (−5.7 to +25.1)	1.48 (0.79–2.76)	0.142
Injectable mineral–vitamin complex	31/41 (75.6)	18/31 (58.0)	+17.6 (−4.2 to +39.3)	2.24 (0.82–6.14)	0.093

Table 1 – Pregnancy rate (%) at natural service of non-pregnant females (Bos indicus) after fixed time artificial insemination according to supplementation type.

4. Discussion

The violin plots presented in Figure 3 (A–B) illustrate the distribution and variability of pregnancy rates following fixed-time artificial insemination (FTAI) in Bos indicus females subjected to hormonal or nutraceutical interventions. The visual symmetry and substantial overlap between the violin densities of treated and control groups clearly indicate that neither long-acting progesterone administration nor injectable micronutrient supplementation exerted a significant influence on conception outcomes. The bootstrap distributions, reinforced by the 95% Wilson confidence intervals, reveal that the observed differences fall within the range of random variation, supporting the absence of treatment effects detected in the statistical models.

The use of long-acting progesterone five days post-FTAI did not translate into improved pregnancy rates (Figure 3A). This finding suggests that the exogenous hormone, while capable of transiently elevating circulating progesterone, did not substantially modify luteal function or uterine receptivity during the early luteal phase. Similar results were reported by Torres et al. (2021) and Bó et al. (2023), who observed that post-insemination progesterone supplementation failed to increase fertility in Bos indicus cows under adequate luteal function. Physiologically, the lack of benefit may be explained by the high endogenous luteal progesterone concentrations typically achieved after synchronized ovulations in cyclic females. Once a functional corpus luteum is established,

additional exogenous progesterone is unlikely to enhance embryo survival or endometrial receptivity beyond the physiological threshold required for maternal recognition of pregnancy (Diskin & Kenny, 2014; Mann & Lamming, 2001).

Conversely, Figure 3B depicts the effect of the injectable mineral–vitamin–amino acid complex administered at the onset of synchronization in heifers. Although the violin shape of the treated group shows a slightly higher central tendency and broader distribution—suggesting greater biological variability—the overlap between groups confirms that the supplementation did not yield a statistically meaningful advantage in pregnancy rate. These results align with prior field studies indicating that short-term parenteral supplementation may not immediately translate into measurable reproductive gains (Rizzo et al., 2019; Arechiga et al., 1998). The reproductive effects of micronutrients are often cumulative and depend on the correction of subclinical deficiencies over time rather than on acute supplementation during a single estrous cycle (Boxmeer et al., 2009; Del Prete et al., 2024).

Nevertheless, the numerical trend toward higher conception rates among supplemented heifers, visible in the violin median shift, may reflect subtle improvements in oxidative balance and follicular competence induced by the nutraceutical formulation. Trace minerals such as zinc, copper, manganese, and selenium act as cofactors for antioxidant enzymes—superoxide dismutase and glutathione peroxidase—that protect granulosa and luteal cells against oxidative stress (Bian et al., 2007; Aurich et al., 2018). Likewise, vitamins A and E, along with β-carotene, enhance follicular vascularization and luteal steroidogenesis, supporting oocyte maturation and embryo viability (Volker & Ludwig, 1995; Boxmeer et al., 2009). Although such metabolic adjustments may require repeated dosing to manifest in reproductive performance, the observed positive tendency corroborates the concept that nutraceutical support contributes to the physiological optimization of the ovarian microenvironment.

Collectively, the visual interpretation of Figure 3 highlights the biological stability of reproductive outcomes across treatments. The absence of statistical significance, combined with overlapping violin densities, underscores that both interventions were physiologically safe—neither disrupting cyclicity nor compromising fertility. From a methodological standpoint, the violin plot approach provides a more nuanced visualization than bar charts or box plots, revealing not only central tendencies but also the shape of the probability distribution and the degree of uncertainty inherent to reproductive data collected under field conditions.

The data summarized in Table 1 complement the violin plots by illustrating the outcomes of the natural mating period that followed FTAI. Among non-pregnant females, neither long-acting progesterone administration nor injectable nutraceutical supplementation produced statistically significant differences in subsequent conception rates ($P > 0.05$). However, the numerical tendency toward higher pregnancy proportions in both treated groups suggest a potential carry-over effect on ovarian function and fertility recovery.

In Experiment 1, cows receiving long-acting progesterone displayed a pregnancy rate of 58.9%, compared with 49.3% in controls, corresponding to an absolute risk difference of +9.7 percentage points and an odds ratio of 1.48. Although these differences were not significant, the trend is biologically plausible. Exogenous progesterone administered after FTAI may contribute to transient endocrine stabilization, supporting the re-establishment of cyclicity and maintenance of luteal sensitivity during the subsequent follicular wave (Mann & Lamming, 1999; Stronge et al., 2005). Nonetheless, as indicated by the similar overall pregnancy rates at 70 days, the hormone did not adversely affect ovulation or corpus luteum formation in the next estrous cycle. This finding reinforces that long-acting progesterone formulations can be safely used in non-pregnant cows without disrupting the hypothalamic–pituitary–ovarian axis, a critical consideration in large-scale reproductive management of Bos indicus herds.

In Experiment 2, heifers supplemented with the injectable mineral–vitamin–amino acid complex achieved a pregnancy rate of 75.6% after natural service, compared with 58.0% in controls (risk difference = +17.6 pp; OR = 2.24). Although the effect did not reach conventional statistical significance (one-sided Fisher's $P = 0.093$), this positive tendency may reflect improved metabolic readiness and oocyte competence induced by the micronutrient formulation. Trace minerals such as selenium, zinc, and manganese enhance antioxidant capacity and steroidogenic efficiency (Bian et al., 2007), while amino acids including methionine and arginine act as precursors for nitric-oxide-mediated follicular vascularization, favoring luteal development (Arechiga et al., 1998; Aurich et al., 2018). Therefore, the observed trend supports the hypothesis that nutraceutical supplementation can modulate the endocrine milieu and facilitate conception during subsequent natural mating, particularly under tropical pasture conditions where latent mineral deficiencies are common.

Taken together, the findings from Table 1 reinforce the interpretation drawn from Figure 3: both interventions were physiologically neutral to beneficial, promoting reproductive stability without inducing endocrine suppression or luteal dysfunction. While the statistical no significance reflects the inherent variability of field data, the consistent direction of effect—favoring treated animals—suggests that combining endocrine support with targeted nutritional supplementation could yield incremental gains in fertility when applied repeatedly or under nutritionally challenged environments. These results warrant further investigation using longitudinal designs and hormonal monitoring to quantify the mechanistic pathways through which nutraceutical and hormonal strategies may converge to optimize post-AI fertility in Bos indicus cattle.

5. Conclusion

In summary, both long-acting progesterone and injectable nutraceutical supplementation were physiologically safe and did not compromise ovarian function or fertility. Although neither treatment significantly increased conception rates, both showed a consistent numerical trend toward improved reproductive performance, suggesting that endocrine and nutritional support strategies may contribute to reproductive stability under tropical field conditions.

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Briefing notes: All experimental procedures were conducted in accordance with institutional guidelines for animal research and were approved by the Ethics Committee on Animal Use (CEUA) of the Federal University of Acre (protocol no. 23107.025574/2018-35).

6. References

Arechiga, C. F., Ealy, A. D., Hansen, P. J., & Thatcher, W. W. (1998). Evidence that glutathione protects preimplantation bovine embryos from heat shock by maintaining embryonic viability and reducing oxidative stress. Biology of Reproduction, 59(5), 1409–1416. https://doi.org/10.1095/biolreprod59.5.1409

Aurich, C., Budik, S., & Aurich, J. E. (2018). Nutritional factors and antioxidant status in relation to fertility in horses. Journal of Equine Veterinary Science, 68, 79–85. https://doi.org/10.1016/j.jevs.2018.04.002

Aurich, C., Budik, S., & Aurich, J. E. (2019). Effects of antioxidant supplementation on reproductive performance in mares. Theriogenology, 132, 169–175. https://doi.org/10.1016/j.theriogenology.2019.04.014

Bian, X., Huang, J., Liang, J., Liu, L., & Li, Y. (2007). Zinc, copper and manganese superoxide dismutase activities and mRNA expression in the bovine corpus luteum. Theriogenology, 68(6), 979–985. https://doi.org/10.1016/j.theriogenology.2007.07.001

Bó, G. A., Cutaia, L., Baruselli, P. S., Menchaca, A., & Mapletoft, R. J. (2023). Advances in reproductive management of beef cattle in South America: Synchronization, embryo technologies, and field fertility. Animal Reproduction, 20(2), e20230012. https://doi.org/10.1590/1984-3143-AR2023-0012

Boxmeer, J. C., Macklon, N. S., Lindemans, J., Beckers, N. G. M., Eijkemans, M. J. C., Laven, J. S. E., Steegers, E. A. P., & Steegers-Theunissen, R. P. M. (2009). Antioxidants and the reproductive system: Impact on sperm, oocytes and embryos. Human Reproduction Update, 15(6), 667–682. https://doi.org/10.1093/humupd/dmp025

CBRA – Colégio Brasileiro de Reprodução Animal. (2013). Manual para Exame Andrológico e Avaliação de Sêmen Animal (3rd ed.). Belo Horizonte: CBRA.

Del Prete, C., Di Palo, R., Salzano, A., & Neglia, G. (2024). Effects of antioxidant vitamin supplementation on reproductive performance and oxidative balance in dairy cows and mares. Animals, 14(3), 455. https://doi.org/10.3390/ani14030455

Diskin, M. G., & Kenny, D. A. (2014). Optimising reproductive performance of beef cows and replacement heifers. Animal, 8(S1), 27–39. https://doi.org/10.1017/S175173111400086X

Mann, G. E., & Lamming, G. E. (1999). The influence of progesterone during early pregnancy in cattle. Reproduction in Domestic Animals, 34(3–4), 269–274. https://doi.org/10.1111/j.1439-0531.1999.tb01250.x

Mann, G. E., & Lamming, G. E. (2001). Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. Reproduction, 121(1), 175–180. https://doi.org/10.1530/rep.0.1210175

Moraes, J. C. F., Hoff de Souza, C. J., Brauner, C. C., Pimentel, M. A., Vieira-Benavides, M., & Sterman-Ferraz, J. B. (2013). Utilização do escore de condição corporal pós-parto na identificação de vacas de corte mais férteis criadas em sistemas extensivos. Archivos Latinoamericanos de Producción Animal, 21(3), 149–155.

Rizzo, A., D'Occhio, M. J., Kaczmarek, M. M., & Williams, S. A. (2019). Nutritional modulation and reproductive efficiency in beef cattle: Impacts on ovarian physiology and endocrine signaling. Animal Reproduction Science, 207, 123–132. https://doi.org/10.1016/j.anireprosci.2019.06.005

Torres, C. A., Pereira, M. H. C., Martins, C. M., & Bó, G. A. (2021). Post-AI progesterone supplementation and fertility in Bos indicus cattle: A meta-analytical perspective. Theriogenology, 170, 1–9. https://doi.org/10.1016/j.theriogenology.2021.04.010

Volker, D., & Ludwig, M. (1995). Role of vitamin A and β-carotene in reproduction. Theriogenology, 43(3), 795–801. https://doi.org/10.1016/0093-691X(94)00022-Y

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Dietary supplementation of *Yucca schidigera* and *Gleditsia amorphoides* modulates gut fermentation metabolites, antioxidant status, and inflammatory markers in adult dogs

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Abstract: Saponins are bioactive compounds known for modulating potential inflammatory metabolites produced by the gut microbiome, including ammonia. This study evaluated the effects of the saponin sources, *Yucca schidigera* (7% saponins) and *Gleditsia amorphoides* (21% saponins), on intestinal functionality, antioxidant, and inflammatory markers in adult dogs. Eighteen Beagle dogs (10 males and eight females), with 12.20 ± 1.33 kg body weight were randomly divided into three groups: Control (no saponins), 200 g *Yucca schidigera*/ton of diet (Yucca), and 200 g *Gleditsia amorphoides*/ton of diet (Gleditsia), totaling six replications per treatment. Dogs were fed experimental diets for 20 days. Fresh fecal samples were collected on day 20 to analyze: dry matter, score, pH, ammonia, short-chain fatty acids (SCFA), branched-chain fatty acids (BCFA), and biogenic amines. Blood was also collected on day 20 to analyze inflammatory cytokines, antioxidant enzymes, and liver enzymes. Preliminary results showed that the Gleditsia group had higher fecal propionate concentrations and lower histamine concentrations ($P < 0.05$). Both the Yucca and Gleditsia groups presented lower fecal concentrations of ammonia and spermine than the Control group ($P < 0.05$). Additionally, the Yucca and Gleditsia groups showed lower lipid peroxidation and higher catalase activity, whereas only the Gleditsia group showed lower alkaline phosphatase activity than the Control group ($P<0.05$). In conclusion, Yucca and Gleditsia can modulate fecal fermentative metabolites and improve the antioxidant status of dogs. These findings also showed the safety of using Yucca and Gleditsia during the experimental period.
Key-words: Ammonia, Propionate, Saponin extracts.

1. Introduction

Saponins, a class of bioactive compounds found in plants, are recognized for their emulsifying properties and modulatory effects on gut microbiome and its metabolites (Zhang et al., 2023). *Yucca schidigera* extract, the most common saponin source in pet food, has demonstrated efficacy in reducing fecal ammonia concentrations and odor in dogs (Dos Reis et al., 2016). However, *Gleditsia amorphoides* has emerged as a promising alternative to yucca due to its higher saponin concentrations (22% vs. 7–15% in yucca) and additional bioactive components, including galactomannans (with prebiotic functions) and polyphenols (with antioxidant activity) (Lu et al., 2024).

Gleditsia amorphoides (Fabaceae family) is a woody species native to temperate and subtropical regions, traditionally valued for its timber and industrial uses (Cerino et al., 2018). Despite its widespread availability and promising properties, only one in vitro study investigating the effects of gleditsia on the modulation of the human fecal microbiome was found (Wang et al., 2023). This study reported a beneficial shift in the fecal microbiome following gleditsia inoculation, characterized by an increase in bacteria with saccharolytic activity and a decrease in those with proteolytic activity. Additionally, functional analysis revealed elevated levels of metabolites linked to antioxidant and anti-inflammatory activities in feces inoculated with gleditsia (Wang et al., 2023). A study investigating the dietary inclusion of a saponin-rich extract (from *Quillaja saponaria*) also observed a significant reduction in fecal proteolytic bacterial populations in dogs (Zhang et al., 2023), thereby supporting the modulatory potential of saponins on the gut microbiome.

Although there is evidence supporting the potential physiological benefits of saponin sources, data on the effects of *Yucca schidigera* and *Gleditsia amorphoides* on gut function and systemic health parameters in dogs remain scarce. To address this knowledge gap, the present study aimed to evaluate the impact of dietary supplementation with *Yucca schidigera* and *Gleditsia amorphoides* on intestinal fermentation metabolites and systemic inflammatory and antioxidant biomarkers in adult dogs.

2. Materials and Methods

The use of animals for this study was approved by the Ethics Committee on Animal Use of the Agrarian Sciences Sector of the Federal University of Paraná, Curitiba, PR, Brazil, under protocol nº. 013/2024. The study was carried out at the Laboratory of Studies in Canine Nutrition (LENUCAN) in Curitiba, Paraná, Brazil (25º 25' 40" S, 49º 16' 23" W).

2.1. Animals and facilities

Eighteen adult neutered/spayed Beagle dogs (10 males and eight females), 2 years of age, with an average body weight of 12.20 ± 1.33 kg and a body condition score of 5 (scale of 1 to 9) were used. All animals underwent prior clinical evaluation and were considered healthy. The researchers and the veterinarian responsible for the laboratory supervised the animals throughout the experimental period. The dogs were individually housed in brickwork kennels (5 m long x 2 m wide), containing a bed and free access to fresh water. During most of the experiment, dogs had free access to an outdoor area of 1.137 m^2 for 5 h/day for voluntary exercise and socialization.

2.2. Experimental Diets

Dogs were randomly assigned to one of three experimental groups (n = 6/group): Control (no saponins), Yucca (200 g/ton *Yucca schidigera* extract), and Gleditsia (200 g/ton *Gleditsia amorphoides,* Sapcor®, Bioaromas do Brasil, Chapecó, SC). The *Yucca schidigera* extract contained at least 7% saponins, and the *Gleditsia amorphoides* extract contained at least 21% saponins, according to the manufacturers of both products. A commercial extruded dry diet formulated for adult dogs was used for the three treatments. Thus, the experimental treatments differ only in the addition or absence of saponin sources, which were included by coating the diet. The commercial diet used contained no functional additives, such as prebiotics, probiotics, or saponins. Its chemical composition was: 22% crude protein, 9.8% ether extract in acid hydrolysis, 3.1% crude fiber, and 9.9% ash.

The animals were fed the experimental diets for 20 days, twice a day (8:00 a.m. and 4 p.m.). The amount of food was calculated based on their metabolizable energy needs for body weight maintenance, as recommended by the NRC (2006). Dogs were weighed weekly, and the amount of food was adjusted as needed.

2.3. Experimental analyses

2.3.1. Fecal analysis

On day 20 of the experiment, fresh fecal samples were collected from each dog to assess fecal characteristics and metabolites of intestinal fermentation. Fecal samples were collected within 15 minutes of defecation and analyzed for dry matter (fDM), fecal score, pH, ammonia, short-chain fatty acids (SCFA - acetate, propionate, and butyrate), branched-chain fatty acids (BCFA – isobutyrate, isovalerate, and 4-metylvalerate), and other volatile fatty acids (VFA – valerate, heptanoic, and hexanoic), and biogenic amines. The fDM was determined at 105°C according to the AOAC recommendations (1995). Fecal score was assessed by a single researcher to ensure standardization, using a scale of 1 (loose stools, no shape) to 5 (dry, hard feces), as described by Carciofi et al. (2009). Fecal pH was measured using a digital pH meter (Politeste, model 331, São Paulo, Brazil) using 3 g of fresh feces diluted in 30 mL of distilled water. Fecal ammonia was determined according to Brito et al. (2010), for SCFA and BCFA, 10 g of feces was mixed with 30 mL of 16% formic acid, homogenized, and stored at 4°C for 3-5 days. After this period, the solutions were centrifuged at 2500 gx (2K15, Sigma, Osterode am Hans, NI, Germany) for 15 min. At the end of centrifugation, the supernatant was separated and subjected to further centrifugation. Each sample underwent three centrifugations, and at the end of the last one, part of the supernatant was transferred to an appropriately labeled Eppendorf tube for subsequent freezing at –14 °C. Afterward, the samples were thawed and subjected to a second centrifugation at 18000 gx for 15 min (Rotanta 460 Robotic, Hettich, Tuttlingen, BW, Germany). Both centrifugations were conducted under refrigeration (approximately 5 °C). The supernatant was collected and analyzed by gas chromatography (Shimadzu GC-2014, Kyoto, Japan) using a glass column (Agilent HP INNO wax, 30 m × 0.32 mm). Nitrogen was used as the carrier gas (3.18 mL/min), with working temperatures of 200°C in the injection, 240°C in the column, and 250°C in the detector. Biogenic amines were quantified as described by Urrego et al. (2017).

2.3.2. Blood analysis

On day 20 of the experiment, blood samples were collected from each dog to assess inflammatory and antioxidant markers and liver enzymes. Blood samples were collected after an 8-hour fasting period. After physical contention and antisepsis with 70% alcohol on the ventral region of the neck, 7 mL of blood was collected by jugular venipuncture. Blood samples for antioxidant and liver enzyme analysis were collected in heparinized syringes and transferred to citrate tubes. In contrast, those intended for cytokine evaluation were collected in syringes without heparin and stored in anticoagulant-free tubes. Cytokines interleukin-6 (IL-6), interleukin-10 (IL-10), and tumor necrosis factor alpha (TNF-α) were quantified by the ProcartaPlex® multiplex immunoassay, which combines ELISA and flow cytometry via Luminex Xmap®. The antioxidant enzymes analyzed were catalase (CAT), glutathione S-transferase (GST), and reduced glutathione (GSH). Lipid peroxidation (LPO) and total antioxidant capacity (T-AOC) were also analyzed. Enzyme activities were determined according to standard methods: CAT (Aebi, 1984), GST (Habig et al., 1974), and GSH (Sedlak & Lindsay, 1968). The LPO was analyzed by the FOX method (Jiang et al., 1991), which quantifies lipid hydroperoxides based on iron oxidation and binding to xylenol orange dye. The T-AOC was analyzed according to Benzie & Strain (1996). Serum alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) activities were measured on an Olympus AU5400 automated analyzer (Olympus America, Center Valley, PA, USA).

2.4. Statistical analyses

Data were analyzed for normality using the Shapiro-Wilk test. Data with normal distribution were subjected to analysis of variance (ANOVA), totaling six repetitions per experimental group. When the ANOVA F-test indicated a difference ($P<0.05$), means were compared by the Tukey test ($P<0.05$). Nonparametric data (cytokine levels) were analyzed using the Kruskal-Wallis test ($P < 0.05$). P-values between 0.05 and 0.10 were considered a tendency.

3. Results

No adverse food reactions, such as vomiting, diarrhea, or food refusal, were observed throughout the study.

3.1. Fecal characteristics

No differences were observed among treatments in fecal score or pH (P > 0.05; Table 1). However, the Gleditsia group showed a tendency toward higher fDM (P=0.076; Table 1).

Item	Control	Yucca	Gleditsia	SEM	P
Dry matter (%)	31.25	31.47	32.74	0.13	0.076
Score	3.83	3.33	3.83	0.11	0.116
pH	6.97	7.24	6.66	0.13	0.197

Table 1 – Means of fecal characteristics of dogs in the Control, Yucca and Gleditsia groups.
Note: SEM: Standard Error of the Mean; P: Probability; Fecal score: 1 = liquid feces to 5 = dry feces.

3.2. Intestinal fermentation metabolites

Yucca and Gleditsia groups had lower fecal ammonia concentrations than the Control group (P < 0.05; Table 2). Fecal propionate and total SCFA concentrations were higher, and 4-methylvalerate was lower in the Gleditsia group than in the Yucca group (P<0.05, Table 2). There was a tendency toward higher fecal acetate concentrations in the Gleditsia group (P=0.057, Table 2). No differences were observed in fecal concentrations of other SCFA and BCFA (P>0.05, Table 2).

Item	Control	Yucca	Gleditsia	SEM	P
Ammonia (%)	0.15a	0.11b	0.10b	0.01	0.012
SCFA (µmol/g)					
Acetate	174.60	130.10	194.60	11.60	0.057
Propionate	50.01b	38.51b	67.93a	3.84	0.001
Butyrate	14.55	12.51	14.63	0.78	0.481
Total SCFA	239.16ab	181.12b	277.16a	15.30	0.024
BCFA (µmol/g)					
Isovalerate	5.64	5.44	5.13	0.10	0.110
Isobutyrate	5.34	5.33	4.98	0.07	0.054
4-methylvalerate	0.55ab	0.63a	0.51b	0.02	0.011
Total BCFA	11.53	11.40	10.62	0.21	0.886
Other VFA ((µmol/g)					
Valerate	5.15	4.99	5.05	0.04	0.244
Heptanoic	5.12	5.02	5.40	0.09	0.239
Hexanoic	1.22	1.19	1.79	0.12	0.068
Total VFA	11.49	11.20	12.24	0.19	0.334

Table 2 – Means (based on dry matter) of fecal concentrations of ammonia, short-chain fatty acids (SCFA), branched-chain fatty acids (BCFA), and other volatile fatty acids (VFA) in dogs from the Control, Yucca and Gleditsia groups.
Note: SEM: Standard Error of the Mean; P: Probability.
[a,b]Means followed by different letters differ according to Tukey's test (P<0.05).

Regarding biogenic amines, fecal concentrations of histamine and spermine were lower in the Gleditsia group than in the Control group (P < 0.05; Table 3). There was a tendency towards lower fecal concentrations of cadaverine (P=0.066) and total biogenic amines (P=0.054) in the Gleditsia group, with no differences in other biogenic amines (P>0.05, Table 3).

Item	Control	Yucca	Gleditsia	SEM	P
Serotonin	31.31	27.24	31.73	1.44	0.369
Tyramine	10.07	5.26	3.08	1.39	0.104
Spermidine	77.55	65.90	76.71	3.86	0.413
Cadaverine	373.90	304.30	145.10	42.10	0.066
Spermine	93.00a	50.19ab	39.84b	8.72	0.019
Histamine	38.35a	37.10a	31.25b	1.07	0.006
Putrescine	676.50	604.60	574.10	34.00	0.479
Total	1301.00	1094.60	901.90	69.5	0.054

Table 3 – Means (mg/kg dry matter) of fecal concentrations of biogenic amines in dogs from the Control, Yucca and Gleditsia groups.
Note: SEM: Standard Error of the Mean; P: Probability.
[a,b]Means followed by different letters differ according to Tukey's test (P<0.05).

3.3. Blood analyses

No significant differences in cytokine levels were observed among treatment groups (P>0.05; Table 4), suggesting that neither *Yucca schidigera* nor *Gleditsia amorphoides* supplementation elicited detectable systemic inflammatory responses.

Item	Control	Yucca	Gleditsia	P
IL-6	18.2 (6,1-69.4)	24.1 (6.1-141.0)	6.1 (6.1-70.7)	0.585
IL-10	6.1 (6.1-76.8)	6.1 (6.1-6.1)	6.1 (6.1-53.04)	0.356
TNF-α	6.1 (6.1-23.50)	6.1 (6.1-65.2)	6.1 (6.1-96.7)	0.885

Table 4 – Medians (minimum-maximum) of cytokines (pg/mL) of dogs in the Control, Yucca and Gleditsia groups.
Note: IL-6: Interleukin-6; IL-10: Interleukin-10; TNF-α: tumor necrosis factor alpha.

There was a reduction in LPO and an increase in CAT in dogs fed diets containing Yucca and Gleditsia, compared with the Control group (P < 0.05; Table 5). There was also a tendency towards higher T-AOC in the Gleditsia group compared to the Control and Yucca groups (P=0.070, Table 5).

Item	Control	Yucca	Gleditsia	SEM	P
LPO	31.96a	26.87b	26.90b	0.922	0.030
GST	8.15	7.28	7.41	0.566	0.827
GSH	66.20	67.31	69.46	0.936	0.380
T-AOC	167.05	168.49	213.63	9.740	0.070
CAT	82.71b	179.29a	157.87a	12.700	0.001

Table 5 – Means of antioxidant system variables of dogs in the Control, Yucca and Gleditsia groups.
Note: SEM: Standard Error of the Mean; P: Probability; LPO: Lipid peroxidation (mmol/mL); GST: Glutathione S-transferase (mmol/min.mL-1); GSH: Reduced glutathione (µM); T-AOC: Total antioxidant capacity (µM trolox equivalent); CAT: Catalase (mU/mL).
[a,b]Means followed by different letters differ by Tukey's test (P<0.05).

No significant differences were observed in AST and ALT enzymes (P>0.05). However, the Gleditsia group exhibited significantly lower ALP activity than the Control group (P < 0.05; Table 6).

Item	Control	Yucca	Gleditsia	SEM	P
ALP	45.10a	37.70ab	33.30b	2.09	0.049
AST	19.75	23.20	24.57	1.33	0.316
ALT	27.90	30.77	30.06	1.32	0.667

Table 6 – Means of liver enzymes (U/L) of dogs in the Control, Yucca and Gleditsia groups.
Note: SEM: Standard Error of the Mean; P: Probabilities; ALP: Alkaline phosphatase; AST: Aspartate amino transferase; ALT: Alanine amino transferase.
[a,b]Means followed by different letters differ by Tukey's test (P<0.05).

4. Discussion

This study demonstrates that dietary supplementation with *Yucca schidigera,* and particularly with *Gleditsia amorphoides,* results in potential benefits for gut function and systemic health in dogs through possible interconnected mechanisms. One of these potential benefits was the increase in fecal propionate concentrations, along with a reduction in fecal concentrations of ammonia, histamine, and spermine in the Gleditsia group compared to the Control group. This modulation of fecal metabolites is likely due to Gleditsia's composition, which combines triterpenoid saponins with galactomannan prebiotics (Lu et al., 2024), thereby collectively shifting fermentation patterns from proteolytic to saccharolytic pathways. Similar in vitro results were obtained using human fecal samples inoculated with *Gleditsia microphylla* extract (Wang et al., 2023). The authors reported modulation of the fecal microbiome and its fermentative activity in these samples, characterized by a decrease in proteolytic bacteria (phylum Fusobacteriota) and an increase in saccharolytic bacteria (phylum Bacteroidota) (Wang et al., 2023).

Propionate, a saccharolytic metabolite, exhibits potential anti-inflammatory effects in the gut by inhibiting the accessory protein CD14 of toll-like receptor 4 (Hoyles et al., 2018). Conversely, proteolytic metabolites such as ammonia and biogenic amines can be toxic to the gut mucosa and liver, with their effects depending on concentration (Brito et al., 2010; Souza et al., 2025). Although no studies have specifically examined the impact of *Gleditsia* spp. in dogs, research on *Yucca schidigera* has demonstrated a reduction in fecal ammonia concentrations in dogs supplemented with this feed additive (Dos Reis et al., 2016). The saponins found in both Yucca and Gleditsia are thought to lower fecal concentrations of proteolytic metabolites through multiple mechanisms, including inhibition of bacterial urease activity, direct binding to these metabolites, and modulation of the gut microbiome (Dos Reis et al., 2016; Zhang et al., 2023).

The potential inflammatory and pro-oxidant effects of proteolytic metabolites in the gut mucosa may also have systemic health implications (Souza et al., 2025). This could partially explain the observed reduction in ALP activity and LPO, alongside the increase

in CAT activity, in dogs from the Gleditsia and Yucca groups. Other studies have similarly reported associations between gut homeostasis and ALP activity across multiple species (Bilski et al., 2017; Salehian et al., 2019), as well as between antioxidant status and ALP activity in dogs (Souza et al., 2025). Beyond modulating the gut microbiome and its activity, Gleditsia may exert additional antioxidant effects through its polyphenol content. Specifically, quercetin derivatives in Gleditsia may enhance the Nrf2 oxidative stress response pathway, as recently demonstrated in canine hepatocyte cultures (Lu et al., 2024). Supporting this, Wang et al. (2023) observed enrichment of antioxidant and anti-inflammatory pathways in functional analyses of human fecal samples inoculated with *Gleditsia microphylla*.

These multi-systemic benefits, especially those observed in the Gleditsia group, suggest that its supplementation holds promise for supporting overall canine health by modulating the gut microbiota metabolites and antioxidant status. However, to enable broader application, further studies are needed to assess long-term impacts and potential interactions across varying dietary compositions and physiological conditions in dogs.

5. Conclusion

The results of this study demonstrate that dietary supplementation with saponins from *G. morphoides* and *Y. schidigera* supports both intestinal and systemic health in dogs, with *G. morphoides* showing the most pronounced effects. The capacity of Gleditsia extract to increase fecal propionate concentrations and reduce ammonia and biogenic amines, along with the enhancement in antioxidant status, highlights its promising potential as a functional additive in canine nutrition.

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6. References

Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology*. 105, 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3

Association of Official Analytical Chemists (AOAC). (1995). *Official methods of analysis* (16th ed.). AOAC International.

Benzie, I. F. F., Strain, J. J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. *Anal. Biochem.* 239, 70–76. https://doi.org/10.1006/abio.1996.0292.

Bilski, J., Mazur-Bialy, A., Wojcik, D., Zahradnik-Bilska, J., Brzozowski, B., Magierowski, M., Mach, T., Magierowska, K., Brzozowski, T. (2017). The role of intestinal alkaline phosphatase in inflammatory disorders of gastrointestinal tract. *Mediators of inflammation*. 9074601, 9. https://doi.org/10.1155/2017/9074601

Brito, C. B. M., Félix, A. P., Jesus, R. M., França, M., Oliveira, S. G., Krabbe, E., Maiorka, A. (2010). Digestibility and palatability of dog foods containing different moisture levels, and the inclusion of a mould inhibitor. *Animal Feed Science and Technology. 159*, 150–155. https://doi.org/10.1016/j.anifeedsci.2010.06.001

Carciofi, A. C., de Oliveira, L. D., Valério, A. G., Borges, L. L., de Carvalho, F. M., Brunetto, M. A., Vasconcellos, R. S. (2009). Comparison of micronized whole soybeans to common protein sources in dry dog and cat diets. *Animal Feed Science and Technology.* 151(3–4), 251–260. https://doi.org/10.1016/j.anifeedsci.2009.01.002

Cerino, M. C., Castro, D. C., Richard, G. A., Exner, E. L., Pensiero, J. F. (2018). Functional dioecy in *Gleditsia amorphoides* (Fabaceae). *Australian Journal of Botany.* 66(2), 85–93. https://doi.org/10.1071/BT16185

Dos Reis, J. S., Zangerônimo, M. G., Ogoshi, R. C. S., França, J., Costa, A. C., Almeida, T. N., Dos Santos, J. P. F., Pires, C. P., Chizzotti, A. F., Leite, C. A. L., Saad, F. M. O. B. (2016). Inclusion of Yucca schidigera extract in diets with different protein levels for dogs. *Animal Science Journal*. 87: 1019–1027. https://doi.org/10.1111/asj.12535.

Habig, W. H., Pabst, M. J., Jakoby, W. B. (1974). Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry.* 249(22), 7130–7139. https://doi.org/10.1016/S0021-9258(19)42083-8

Hoyles, L., Snelling, T., Umlai, U. K., Nicholson, J. K., Carding, S. R., Glen, R. C., McArthur, S. (2018). Microbiome–host systems interactions: protective effects of propionate upon the blood–brain barrier. *Microbiome*. 6, 55. https://doi.org/10.1186/s40168-018-0439-y

Jiang, Z. Y., Woollard, A. C. S., Wolff, S. P. (1991). Lipid hydroperoxide measurement by oxidation of Fe^{2+} in the presence of xylenol orange: Comparison with the TBA assay and an iodometric method. *Lipids.* 26(10), 853–856. https://doi.org/10.1007/BF02536169

Lu, G., Ren, T., Zhao, Z., Li, B., Tan, S. (2024). Chemical component differences in the endosperm of *Gleditsia* species seeds revealed based on comparative metabolomics. *Food Chemistry: X, 21*, 101060. https://doi.org/10.1016/j.fochx.2023.101060

National Research Council (NRC). (2006). *Nutrient requirements of dogs and cats*. National Academies Press.

Sedlak, J., Lindsay, R. H. (1968). Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Analytical Biochemistry. 25*, 192–205. https://doi.org/10.1016/0003-2697(68)90092-4

Salehian, P., Shareghi, B., Hosseini-Koupaei, M. (2019). Comparative studies on the interaction between biogenic polyamines and bovine intestinal alkaline phosphatases: spectroscopic and theoretical approach. *Journal of Biological Physics*. 45:89-106. https://doi.org/10.1007/s10867-018-9517-4

Souza, R. B. M. S., Fernandes, E. L., Santos, L. N. A., Lima, L. S., Silva, H. L., Putarov, T. C., Oliveira, S. G., Felix, A. P. (2025). Effects of yeast beta-1,3/1,6-glucans on nutrient digestibility, intestinal functionality, and immune and antioxidant variables in growing dogs submitted to spay or neutering surgery. *Plos One*. 20(9): e0331843. https://doi.org/10.1371/journal.pone.0331843

Urrego, M. I. G., Matheus, L. F. O., de Melo Santos, K., Ernandes, M. C., Monti, M., de Souza, D. F., Balieiro, J. C. C., Araújo, L. F., Pontieri, C. F. F., Brunetto, M. A. (2017). Effects of different protein sources on fermentation metabolites and nutrient digestibility of brachycephalic dogs. *Journal of Nutritional Science.* 6, e43. https://doi.org/10.1017/jns.2017.46

Wang, H., Lai, C., Tao, Y., Zhou, M., Tang, R., Yong, Q. (2023). Evaluation of the enzymatic production and prebiotic activity of galactomannan oligosaccharides derived from *Gleditsia microphylla*. *Fermentation*. 9(7), 632. https://doi.org/10.3390/fermentation9070632

Zhang, Y., Wang, L., Chen, X., Liu, Y., Li, J. (2023). *Quillaja saponaria* extract modulates gut microbiota and reduces proteolytic bacteria in dogs: A dose-response study. *Veterinary Microbiology.* 276, 109634. https://doi.org/10.1016/j.vetmic.2023.109634

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Rate of umbilical healing in newborn piglets: the influence of neonatal practices and birth weight

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Abstract: This study evaluated the effects of different umbilical cord care techniques in newborn piglets. A total of 212 piglets from 15 sows were allocated in a randomized block design. Treatments included: No Intervention (NI) – no intervention was performed in the cord at birth; Tying and Cutting (TC) – the cord was tied onto itself and cut below the knot; and String, Cutting, and Iodine (SCI) – the cord was tied with string, cut, and immersed in 10% iodine solution. The following variables were analyzed: colostrum intake, hemorrhage incidence, healing time, umbilical hernia incidence, growth performance. No treatment effects were found for colostrum intake, performance, hemorrhage incidence, or umbilical hernia occurrence. Piglets in the TC group showed a slightly shorter healing time than those in the NI and SCI groups (P = 0.035). Birth and weaning weights were negatively correlated with healing time—lighter piglets exhibited slower healing, whereas faster-healing piglets achieved higher weaning weights. In conclusion, additional umbilical care, such as iodine application, did not improve umbilical healing and performance. Under the conditions of this study, additional procedures, such as iodine application, did not confer additional benefits over a simple tying technique.
Keywords: Hernia, Pre-Weaning, Sus scrofa domesticus, Swine, Umbilical Outpouchings.

1. Introduction

In swine production systems, implementing management practices tailored to each production stage is essential to achieve optimal performance and reduce both productive and economic losses. The suckling phase, spanning from birth to weaning (typically 21-28 days of age), is considered one of the most critical stages of the production cycle, as it is associated with the highest mortality rate (Davidov et al., 2024). Previous research has shown that management practices during the pre-weaning period can influence performance traits, immune system development, and even meat quality later in life, ultimately impacting the overall profitability of pig production (Kwon et al., 2025). Given the vulnerability of neonatal piglets, producers place great emphasis on specific management practices — both preventive and therapeutic — implemented immediately after birth. These practices include ensuring thermal comfort and adequate colostrum and milk intake, as well as conducting health interventions such as iron supplementation, anticoccidial treatments, and vaccinations (Kumar et al., 2025).

Among these practices, umbilical cord management is particularly relevant. The umbilical cord connects the fetus to the placenta, enabling the transfer of nutrients and the elimination of waste during gestation (Manuel Barrios Arpi, 2019). It comprises essential vessels, such as the umbilical vein, the arteries, and the urachus, which connect the placenta to the liver, the aorta, and the bladder, respectively (Pinheiro et al., 2024). After birth, these vessels are expected to dry and close naturally, severing these connections. However, delayed healing can result in umbilical outpouchings (UO), which are primarily caused by hernias or abscesses with associated fibrosis (Hovmand-Hansen et al., 2021a).

In commercial farms, this procedure commonly includes tying the cord with a string, cutting it, and disinfecting it with an iodine-based solution (Robinson et al., 2016). Nevertheless, recent studies have questioned whether alternative, more effective approaches to umbilical cord care exist or whether such management is even necessary in farms with high sanitary standards and low environmental contamination (Hansen et al., 2024; Robinson et al., 2016). Therefore, the objective of this study was to compare different umbilical cord care management techniques applied at birth and evaluate their effects on piglets' colostrum intake, incidence of hemorrhage, healing time, incidence of umbilical hernias throughout the productive lifespan, and growth performance.

2. Materials and Methods

This experiment was approved by the Ethic Committee on Animal Use (CEUA) of the School of Veterinary Medicine and Animal Science (FMVZ) of the University of São Paulo (USP) (CEUA N° 4899180325). The study was conducted on the Swine Research Laboratory (LPS) at FMVZ/USP, located in Pirassununga (21°56'56.9" S 47°27'16.1" W), São Paulo, Southeastern Brazil.

2.1. Animals, Facilities, and Experimental Design

A total of 212 piglets from 15 third-parity sows (DB 90 line) crossed with LQ1250 boar, were used. The experiment was carried out in a conventional farrowing facility with curtain, controlled ventilation, and no automatic climate control. Thermal comfort for piglets was provided by 100 W heat lamps and heated floors in the creep area. Piglets in all treatments were dried using drying powder (Olmix, Campinas, Brazil) at birth and individually identified with ear tags. All pens were equipped with nipple drinkers for the piglets, and creep feed was provided from the seventh day of life until weaning. The sows were in good general health and body condition at farrowing, and all procedures were performed under the same sanitary management conditions adopted by the experimental farm. The experimental treatments consisted of: No Intervention (NI) - no intervention was performed on the umbilical cord; Tying and Cutting (TC) - the umbilical cord was tightly tied onto itself and cut below the tied area and; String, Cutting and Iodine (SCI) – the umbilical cord was tied with string, cut below the tied area, and immersed in 10% iodine. In the TC and SCI treatments, the umbilical cord was tied approximately 5 cm away from the piglet's abdominal wall.

2.2. Performance and umbilical cord analysis

Piglets were individually weighed at birth, at 24 hours of age, and at weaning using a digital scale (Welmy, Santa Bárbara d'Oeste, Brazil). Colostrum intake was estimated according to the predictive model described by Theil et al. (2024). To assess the occurrence of hemorrhages, piglets were observed twice daily from birth until the umbilical cord had healed and detached. Hemorrhage was classified as either positive (occurrence of hemorrhage in the cord) or negative (absence of hemorrhage in the cord). To determine the number of days required for complete healing and umbilical cord detachment, all piglets were evaluated daily. Hernia occurrence was recorded during the pre-weaning phase (0 to 21 days), the nursery phase (21 to 63 days), and the grow-finishing phase (63 to 167 days). The same experienced veterinarian performed all evaluations.

2.3. Statistical analysis

Data were analyzed using R software (version 4.2.1; R Core Team, Vienna, Austria). Normality and homoscedasticity were assessed both visually and through the Shapiro-Wilk and Bartlett tests, respectively. Performance data that did not meet the normality assumption were transformed using the Box-Cox method. Data were analyzed using mixed-effects models, with sow included as a random effect. Differences in mean values were considered significant at $P < 0.05$, and means were compared using the Tukey test. For umbilical outpouching, hemorrhages, and umbilical cord detachment, multivariable regression was performed using a stepwise forward selection approach. Logistic regression was used to analyze incidence data. Initially, each independent variable was tested in univariate models, and those with p-values ≤ 0.20 were selected as candidates for inclusion in the multivariable model. Variables were then sequentially added and removed based on their statistical contribution, with model selection guided by the lowest Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) values.

Throughout the model-building process, multicollinearity was assessed using the Variance Inflation Factor (VIF). Variables with VIF values above 5 were considered indicative of high collinearity and were carefully evaluated for removal to ensure model stability and interpretability. Final regression models only include variables with $P < 0.1$. Additionally, two Principal Component Analyses (PCA) were performed. Individuals were colored by experimental treatment, with 95% confidence ellipses computed around treatment centroids. Variable contributions were visualized using factor coordinates, and multivariate group differences were statistically tested via PERMANOVA.

3. Results

The different umbilical cord management techniques applied at birth did not affect body weight at 24 h, weight gain during the first 24 h, or pre-weaning weight (Table 1).

Variables	NI	TC	SCI	SEM	P-Value
Birth weight, kg	1.277	1.289	1.249	0.047	0.525
Weight at 24 hours, kg	1.286	1.314	1.264	0.047	0.507
Weight gain 0-24h, kg	0.009	0.021	0.016	0.015	0.860
Weaning weight, kg	6.658	7.033	6.755	0.359	0.552
Colostrum intake, g	314.038	310.373	306.873	18.358	0.936

NI: No Intervention group. **TC:** Tying and Cutting group. **SCI:** String, Cutting and Iodine group. **SEM:** Standard Error of the Mean.

Table 1 – Growth performance and colostrum intake of piglets submitted to different umbilical cord management approaches.

The results of the univariate analysis showed that treatment, birth weight, birth order, and sex did not influence the incidence of hemorrhage or umbilical hernias ($P > 0.20$). For umbilical healing, the univariate analysis indicated significant effects of treatment ($P = 0.035$), birth weight ($P = 0.016$), sex ($P = 0.077$), and colostrum intake ($P = 0.045$). However, sex ($P = 0.331$) and colostrum intake ($P = 0.380$) were excluded from the multivariate analysis, and the final model included treatment ($P = 0.051$) and birth weight ($P = 0.026$, supplementary material). The incidence of hemorrhage and umbilical hernias was not affected by the treatments (Table 2). However, piglets in the TC group had a shorter interval between umbilical cord management and healing time (- 7.25%) than

those in the NI and SCI groups (P = 0.035). Of the 16 umbilical hernias observed in this study, one (6.25%) was observed in the nursery phase, while the others were manifested in the finishing phase.

Variables	NI	TC	SCI	SEM	P-Value
Occurrence of hemorrhage, %	2.53	8.19	6.94	3.51	0.336
Interval between birth and umbilicus dropping, days	3.45a	3.20b	3.45a	0.135	**0.035***
Incidence of umbilical hernia, %	7.24	6.78	11.15	4.65	0.631

NI: No Intervention group. **TC:** Tying and Cutting group. **SCI:** String, Cutting and Iodine group. **SEM:** Standard Error of the Mean. Mean followed by different letters differ significantly between the treatments, using Tukey's test (α=0.05).

Table 2 – Occurrence of hemorrhage, umbilicus drop time and incidence of umbilical hernia of piglets submitted to different umbilical cord management approaches.

The PCA plot (Figure 1) showed that the first principal component accounted for 38.9% of the total variance, while the second principal component accounted for 21.3%. Both birth weight and weaning weight were negatively correlated with umbilical cord healing time, indicating that piglets with lower birth weights required longer umbilical cord healing. In contrast, those with faster umbilical cord healing achieved higher weaning weights. Birth weight was positively correlated with weaning weight and colostrum intake.

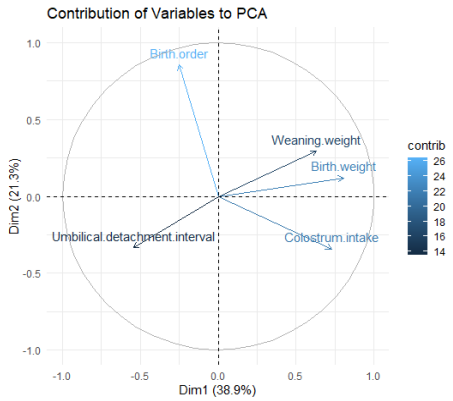

Figure 1 – Principal component analysis (PCA) plot showing the contribution of variables birth order, weaning weight, birth weight, umbilical detachment interval and colostrum intake of piglets submitted to different umbilical cord management approaches.

Figure 2 shows the distribution of the first (38.9%) and second (21.3%) principal components, which together explain 60.2% of the total variance in the treatment-related data. Although there was overlap among the three treatment groups, indicating limited overall separation, the TC group exhibited a subtle but consistent directional shift.

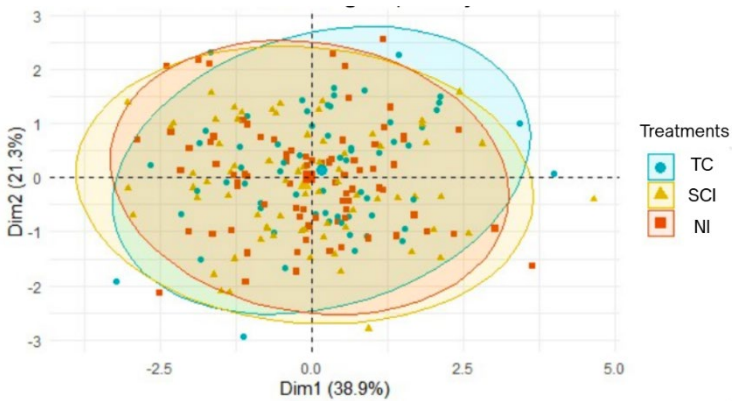

Figure 2 – Principal component analysis (PCA) plot showing the contribution of the treatments to the variables. **NI:** No Intervention group. **TC:** Tying and Cutting group. **SCI:** String, Cutting and Iodine group.

4. Discussion

Birth weight and early growth are fundamental indicators of piglet viability and performance potential. These variables are influenced, but not limited to, by litter size, colostrum intake, sow health status, and genetic background (Farmer and Edwards,

2022). Piglets from large litters generally exhibit greater birth weight variability, which can hinder access to colostrum and reduce weight gain on the first day of life (Quesnel et al., 2023). Heavier piglets tend to be more vigorous, initiating suckling earlier and obtaining higher amounts of colostrum, which supports passive immunity and subsequent growth (Milligan et al., 2001; Nuntapaitoon et al., 2019).

In the present study, although no significant differences in performance were observed among treatments, a positive correlation was identified between birth weight, colostrum intake, and weaning weight. This reinforces the role of initial body weight as a predictor of postnatal development. Similar relationships have been described by Kwon et al. (2025), who reported that heavier piglets at weaning show improved growth and feed efficiency in the nursery phase. The absence of differences among groups suggests that none of the evaluated umbilical management practices impaired piglet performance, a relevant result from a practical standpoint, as it indicates that simpler handling practices did not negatively affect productivity. Contrary to expectations, no association between colostrum intake and healing time was observed, suggesting that the environmental and individual factors may exert greater influence. Both systemic and local factors influence the processes of umbilical and wound healing (Martin et al., 2016). Therefore, although passive immunity may indirectly contribute to healing by reducing the risk and severity of infection during this period, its effect is likely to be secondary.

In this study, birth and weaning weights were negatively correlated with healing time, indicating that lighter piglets required longer to heal the umbilicus. This could be explained by the fact that piglets with low birth weight may exhibit impaired transfer of antibodies and neonatal immune cells, which negatively affects both the initial inflammatory response and tissue regeneration mechanisms, thereby directly interfering with umbilical cord healing. This finding can be explained by the observations of Hovmand-Hansen et al. (2021a), who reported that piglets born immature are more likely to develop UO due to the immature structure of their tissues. Additionally, lighter piglets often experience difficulties with thermoregulation, which can further compromise cellular metabolism involved in healing processes (Devillers et al., 2011; Ferrari et al., 2014). Prolonged healing may increase vulnerability to infection because the exposed umbilical stump remains a potential entry point for pathogens (Barington et al., 2024; Blirup-Plum et al., 2025). Environmental hygiene and thermal conditions also affect healing rates, as contaminated or cold environments can delay cord drying and increase bacterial colonization (Schokker et al., 2014).

Umbilical hernias are an important welfare and economic concern, being associated with carcass condemnation, secondary infections, intestinal torsions, and, in severe cases, mortality (Straw et al., 2009; Yun et al., 2017; Hovmand-Hansen et al., 2021b; Nowacka-Woszuk, 2021). Different umbilical cord management strategies did not affect the incidence of hernias. This result was expected, primarily due to the low incidence of umbilical hernias, but also considering that factors reported as important for hernia development, such as pre-weaning weight gain, early immunity (acquired through colostrum intake), and the presence of infections during the pre-weaning period, did not differ among treatments (Hovmand-Hansen et al., 2021a; Searcy-Bernal et al., 1994). Previous studies have reported that delayed cord drying may favor hernia formation by maintaining local humidity and promoting bacterial growth (Hovmand-Hanses et al., 2021b). In a study by Hovmand-Hansen et al. (2021b), it was reported that more than half of pigs with hernias or other umbilical lesions either died or were euthanized before reaching slaughter weight, directly impacting productivity.

Although the faster healing observed in the TC group should be interpreted with caution, given the small effect size, it remains an interesting result. It may serve as a starting point for future studies on UO prevention. Moreover, although the farrowing phase accounts for only 15–20% of the piglets' lifespan, it is the period with the highest mortality rate (Davidov et al., 2024), underscoring the importance of even small management practices to reduce losses.

All management practices implemented in commercial pig farms require both resources and labor. In the present study, additional umbilical care practices—such as cord tying or iodine application—were not effective in preventing umbilical hernias, suggesting that such practices may be unnecessary. It is important to note that genetic factors, environmental conditions, and individual characteristics of sows and piglets may influence these outcomes. Therefore, the specific context of each farm should be carefully evaluated before adopting or discontinuing disease prevention strategies. The results also emphasize the strong influence of birth weight on healing and performance, underscoring the importance of farrowing management strategies to improve neonatal vitality and uniformity.

5. Conclusion

Under the conditions of this study, adopting additional umbilical care practices, such as tying with a string and applying iodine solution, was not effective in reducing hemorrhage, shortening healing time, preventing umbilical hernias, or improving productive parameters. Simply tying the umbilical cord to itself significantly accelerated healing. Overall, the findings highlight that birth weight plays a greater role than umbilical cord treatment in influencing healing time and early performance. The data emphasize the need for management practices that improve piglet vitality and uniformity at birth.

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6. References

Arpi, LMB. Histology of the umbilical cord in mammals. In: Histology [Internet], 2019. Available in: http://dx.doi.org/10.5772/intechopen.80766. Access in: 7 jul. 2025.

Blirup-Plum SA, Jensen HE, Hartmann KT, et al. Can infectious omphalitis in piglets be clinically diagnosed during the first three days of life? BMC Vet Res, v. 21, p. 58, 2025. https://doi.org/10.1186/s12917-025-04507-3

Cabrera RA, Lin X, Campbell JM, et al. Influence of birth order, birth weight, colostrum and serum immunoglobulin G on neonatal piglet survival. J Anim Sci Biotechnol, v. 3, n. 1, p. 42, 2012. https://doi.org/10.1186/2049-1891-3-42

Davidov I, Stevančević O, Božić A, et al. Performance comparisons across pig farms. Biotechnol Anim Husb, v. 40, n. 2, p. 131–140, 2024. https://doi.org/10.2298/BAH2402131D

Devillers N, Dividich JL, Prunier A. Influence of colostrum intake on piglet survival and immunity. Animal, v. 5, n. 10, p. 1605–1612, 2011. https://doi.org/10.1017/S175173111100067X

Farmer C, Edwards SA. Review: Improving the performance of neonatal piglets. Animal, v. 16, n. 2, p. 100350, 2022. https://doi.org/10.1016/j.animal.2021.100350

Ferrari CV, Sbardella PE, Bernardi ML, et al. Effect of birth weight and colostrum intake on mortality and performance of piglets after cross-fostering in sows of different parities. Prev Vet Med, v. 114, n. 3–4, p. 259–266, 2014 https://doi.org/10.1016/j.prevetmed.2014.02.013

Hansen ML, Larsen I, Jensen TB, et al. Prevention of umbilical outpouchings and mortality in pigs. Porcine Health Manag, v. 10, p. 10, 2024. https://doi.org/10.1186/s40813-024-00358-w

Hovmand-Hansen T, Jensen TB, Vestergaard K, et al. Early risk factors, development, disappearance and contents of umbilical outpouching in Danish pigs. Livest Sci, v. 251, p. 104654, 2021a. https://doi.org/10.1016/j.livsci.2021.104654

Hovmand-Hansen T, Nielsen SS, Jensen TB, et al. Survival of pigs with different characteristics of umbilical outpouching in a prospective cohort study of Danish pigs. Prev Vet Med, v. 191, p. 105343, 2021b. https://doi.org/10.1016/j.prevetmed.2021.105343

Juthamanee P, Tummaruk P. Factors associated with colostrum consumption in neonatal piglets. Livestock Science, v. 251, p. 104630, 2021. https://doi.org/10.1016/j.livsci.2021.104630

Kumar N, Thakur R, Harini KR, et al. Care and management of piglets. In: Commercial pig farming. p. 173–184, 2025. https://doi.org/10.1016/B978-0-443-23769-0.00011-7

Kwon CH, Safaie ES, Torres JA, et al. Effects of pigs' weaning weight on growth performance and blood immunological, antioxidant, and gut permeability parameters in early nursery period. Animals, v. 15, n. 8, p. 1119, 2025. https://doi.org/10.3390/ani15081119

Martin YH, Lali FV, Metcalfe AD. Modelling wound healing. In: MS Ågren, editor. Wound healing biomaterials. Cambridge: Elsevier, 1: 151–73, 2016. https://doi.org/10.1016/B978-1-78242-455-0.00006-9

Matheson SM, Walling GA, Edwards SA. Genetic selection against intrauterine growth retardation in piglets: a problem at the piglet level with a solution at the sow level. Genet. Sel. Evol., v. 50, p. 46, 2018. https://doi.org/10.1186/s12711-018-0417-7

Milligan B, Fraser D, Kramer D. The effect of littermate weight on survival, weight gain, and suckling behavior of low-birth-weight piglets in cross-fostered litters. J of Swine Health Prod, v. 9, n. 4, p. 161–166, 2001. http://dx.doi.org/10.54846/jshap/302

Nowacka-Woszuk J. The genetic background of hernia in pigs: A review. Livest Sci, v. 244, p. 104317, 2021. https://doi.org/10.1016/j.livsci.2020.104317

Nuntapaitoon M, Muns R, Theil PK, et al. Factors influencing colostrum consumption by piglets and their relationship with survival and growth in tropical climates. Livest Sci, v. 224, p. 31–39, 2019. https://doi.org/10.1016/j.livsci.2019.04.008

Pinheiro JM, Ferraz RHS, Ribeiro M, et al. Fetal external features and morphology of the umbilical cord of wild boars. Anat Histol Embryol, v. 53, n. 3, p. e13041, 2024. https://doi.org/10.1111/ahe.13041

Quesnel H, Resmond R, Merlot E, et al. Physiological traits of newborn piglets associated with colostrum intake, neonatal survival and preweaning growth. Animal, v. 17, n. 6, p. 100843, 2023. https://doi.org/10.1016/j.animal.2023.100843

Robinson AL, Colpoys JD, Robinson GD, et al. The effect of antiseptic compounds on umbilical cord healing in piglets in a commercial facility. J of Swine Health Prod, v. 24, n. 4, p. 212–215, 2016. https://doi.org/10.54846/jshap/954

Searcy-Bernal R, Gardner IA, Hird, DW. Effects of and factors associated with umbilical hernias in a swine herd. JAVMA, v. 20, n. 204, 1994. https://doi.org/10.2460/javma.1994.204.10.1660

Schokker D, Zhang J, Zhang L, et al. Early-life environmental variation affects intestinal microbiota and immune development in newborn piglets. PLoS One, v. 9, n. 6, p. e100040, 2014. https://doi.org/10.1371/journal.pone.0100040

Straw B, Bates R, May G. Anatomical abnormalities in a group of finishing pigs: prevalence and pig performance. J of Swine Health Prod, v. 17, n. 1, p. 28–31, 2009. http://dx.doi.org/10.54846/jshap/578

Theil PK, Flummer C, Hurley WL, et al. Mechanistic model to predict colostrum intake based on deuterium oxide dilution technique data and impact of gestation and pre-farrowing diets on piglet intake and sow yield of colostrum. J Anim Sci, v. 92, n. 12, p. 5507–5519, 2014. https://doi.org/10.2527/jas.2014-7841

Yun J, Olkkola S, Hänninen ML, et al. The effects of amoxicillin treatment of newborn piglets on the prevalence of hernias and abscesses, growth and ampicillin resistance of intestinal coliform bacteria in weaned pigs. PLoS One, v. 12, n. 2, p. e0172150, 2017. https://doi.org/10.1371/journal.pone.0172150

Zheng L, Duarte ME, Loftus AS, et al. Intestinal health of pigs upon weaning: Challenges and nutritional intervention. Front Vet Sci, v. 8, p. 628258, 2021. https://doi.org/10.3389/fvets.2021.628258

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