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The Impact of Age at First Calving on First Lactation Performance in Dairy Cows

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Keywords: dairy cows, age at first calving, first lactation, productivity.

Abstract. The subject of this study is a dairy cattle farm with a capacity of 500 Holstein cows. The housing system is a free-stall group system with individual resting stalls. The study included all cows present on the farm as of April 2024 – a total of 425 animals. Based on individual animal records, the age at first calving and the cows' age as of April 2024 were calculated. The average age at first calving in the studied herd was within the optimal and recommended range – 716.26 days (23.88 months), with a high average first-lactation milk yield of 11 495.21 kg. A substantial variation in the age at first calving was observed depending on year of birth and corresponding lactation number. Only 1.18% of the cows calved for the first time before the age of 660 days. The majority of the cows – 90.35% – had their first calving between 22 and 26 months of age. Only 8.47% calved for the first time after 780 days of age. The variation in age at first calving across lactation numbers suggests that insemination of heifers on this farm typically begins after they reach one year of age. The lowest average first-lactation milk yield was recorded in cows that calved before the age of 659 days. In contrast, the highest average first-lactation milk yield was observed in cows that calved for the first time at over 27 months of age – 12 025.54 kg.

Introduction

The issue of optimizing the age at first calving (AFC) in dairy cows remains a challenge in many countries worldwide. The considerable interval between birth and first calving makes it difficult for most dairy producers to assess the impact of their heifer-rearing strategies on both individual animals and overall farm performance (Mourits et al., 2000). According to these authors, the importance of heifers in dairy production is not sufficiently recognized and is often misinterpreted by farmers.

Determining the optimal time for insemination in heifers is complex, as it must ensure the full utilization of their genetic potential for high productivity. On the one hand, economic considerations are of great importance, while on the other, the physiological status and health of first-calving cows are crucial. According to Levina et al. (2019), the physiological characteristics of heifers must be carefully evaluated prior to breeding. A thorough understanding of the factors affecting milk production efficiency can reduce costs and improve productivity.

In their study, Fodor et al. (2020) found that both very early and delayed insemination of heifers negatively affect milk production. Age at first calving is a key factor strongly influencing milk yield and milk composition (Pirlo et al., 2000; Petraskiene et al., 2007; Salomończyk and Guliński, 2010), reproductive performance (Evans et al., 2006), and longevity (Ojango et al., 2005). Reducing AFC also contributes positively to genetic progress by shortening

the generation interval and enabling earlier progeny testing of young bulls. Conversely, later breeding of heifers results in higher rearing costs (Turiello et al., 2020). Extending AFC beyond 750 days does not improve lactation performance, reproductive traits, or health status (Ettema et al., 2004).

Vargas-Leitón et al. (2023) reported that AFC significantly affects the profitability of dairy farms, noting that a heifer typically does not become profitable until her second lactation. Earlier AFC may reduce rearing costs due to lower feed and labor requirements (Tozer and Heinrichs, 2001). Similarly, Kusaka et al. (2023) suggest that early breeding of heifers may lead to increased profitability.

The average age at first breeding in Holstein heifers has decreased by nearly three months – from 28.6 to 25.7 months – clearly indicating a trend among farmers toward reducing AFC (Fodor et al., 2018).

However, current data on the impact of AFC on subsequent dairy cow performance are not always consistent. These discrepancies may partly result from differences in genetics, management practices, feeding, and other factors across studies and countries (Shook, 2006).

Berezkina et al. (2020) demonstrated that age at first insemination has a significant effect on milk yield, reproductive performance, health status, and postpartum complications in heifers. Proper farm management can improve the economic efficiency of animal utilization without compromising reproductive performance or adversely affecting milk productivity, even with early breeding (Kepkalo et al., 2024).

Therefore, farmers require precise and evidence-based information to support informed management

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decisions regarding heifer growth rates and rearing strategies.

The aim of this study is to assess reproductive performance, specifically age at first calving, and its association with subsequent milk yield in a high-producing dairy farm in Bulgaria over recent years.

Materials and Methods

The study was conducted in a commercial dairy farm located in the central part of Southern Bulgaria. The farm has a capacity of 500 Holstein dairy cows. The animals are housed in free-stall group pens with individual cubicles for resting. Milking is performed twice daily in a "herringbone" milking parlor. Cows are fed ad libitum with a total mixed ration (TMR), formulated according to their physiological status and level of milk production.

The study included all cows present on the farm as of April 2024 specifically, those born between 2014 and 2022, amounting to a total of 425 animals. Of these, 329 cows had completed at least one normal lactation. Data on pedigree, birth dates, calving dates, and milk yield for all completed lactations were obtained from the farm's records. The cows included in the analysis were daughters of 29 different sires.

Age at first calving and age of the animals as of April 2024 were calculated based on individual animal data. For better approximation, AFC was used in the statistical models as a fixed factor categorized into six classes: 1 – up to 659 days; 2 – 660 to 689 days; 3 – 690 to 719 days; 4 – 720 to 779 days; 5 – 780 to 809 days; and 6 – over 810 days.

Basic statistical analyses were performed using appropriate modules from the software packages MS Excel and STATISTICA (StatSoft Inc.).

To examine the influence of controlled factors on age at first calving, the following linear model was applied:

$$Y_{ijk} = \mu + G_i + Sire_j + e_{ijk},$$

where Y_{ijk} is the dependent variable (age at first calving), μ is the overall mean, G_i is the fixed effect of year of birth, $Sire_j$ is the fixed effect of sire, e_{ijk} is the effect of random residual variation not explained by the fixed effects or the overall mean (μ).

To assess the influence of the studied factors on first-lactation milk yield, the following linear model was used:

$$Y_{ijklm} = \mu + G_i + Ag_j + Lk + Sire_l + e_{ijklm},$$

where Y_{ijklm} is the dependent variable (milk yield in first lactation), μ is the population mean, G_i is the

fixed effect of year of birth, Ag_j is the fixed effect of AFC class, Lk is the fixed effect of lactation number reached, $Sire_l$ is the fixed effect of sire, e_{ijklm} is the effect of random residual variation not explained by the fixed effects or the overall mean (μ).

Analysis of variance (ANOVA) was used to obtain least square means (LSM) for the fixed factor classes in both models. These LSM represent the sum of squares calculated as deviations from the model-derived mean value of the respective trait.

Results and Discussion

Table 1 presents the mean values and variation of the studied traits in the herd. The average age at first calving for all cows present at the time of the study ($n = 425$) was 716.26 days (equivalent to 23.88 months), with considerable individual variation from 655 to 872 days, or 21.83 to 29.07 months, respectively. The average age of all lactating cows in the herd was 1456.58 days, or 3.99 years, ranging from young primiparous cows under two years of age (681 days) to nearly 10 years (3600 days).

By subtracting the mean AFC from the average age of the cows, it is evident that the average productive lifespan of the current herd is slightly over two years, which corresponds to approximately two lactations.

The average milk yield per lactation was calculated only for cows that had completed a lactation of normal duration (between 240 and 305 days), comprising 329 animals. The remaining 96 cows were primiparous and still in lactation but had not yet reached 240 days as of April 2024.

The mean milk yield was 11 966.17 kg, with values ranging from 5143.18 kg (typically among first-lactation cows) to 22 690.12 kg in cows at later lactations.

In order to maximize economic efficiency in dairy farming, several studies suggest that the optimal age at first calving should range between 23 and 24.5 months (Pirlo et al., 2000; Ettema and Santos, 2004). Achieving this target is considered feasible at a population level, provided that proper management and conditions are in place. However, in some countries, the average AFC remains higher. For instance, the mean AFC for Holstein cows is 26.5 months in the Canadian province of Quebec (Pietersma et al., 2006); 28 months in Sweden (Wejdmark, 2014); 30.7 months in Egypt (Hammoud et al., 2010); and 29.5 months in Honduras (Vargas-Leitón et al., 2023).

Table 1. Mean values and variation of the studied traits in the farm

Trait	Number	$\bar{x} \pm Se$	SD	min	max
AFC, days	425	716.26 \pm 1.912	39.427	655.0	872.0
Age, day	425	1456.58 \pm 28.001	377.263	681	3600
Milk yield, kg	329	11 966.17 \pm 168.41	3054.71	5143.18	22 690.12

Note: AFC= age at first calving.

In Bulgaria, data from previous years indicate that AFC has historically been higher than the recommended range. Gergovska and Yordanova (2011) reported an average AFC of 892.1 days (29.7 months) for 1,460 Black-and-White cows calving during the period 1995–2006. Lower values were reported by Penev et al. (2014) – 26.6 months in seven farms. Valchev et al. (2020) found an average AFC of 29.68 months across five farms in Southern Bulgaria, with values ranging from 28.79 to 30.33 months.

In a study conducted on 1200 cows, Siriak et al. (2022) found that when the AFC was under 22 months or over 34 months, a persistent trend toward reduced productive lifespan and overall productivity of the animals was observed.

In the present study, the evaluated farm exhibited a notably lower AFC of 716.26 days or 23.88 months, which falls within the internationally recommended range. This outcome indicates an effective reproductive strategy, high-quality feeding, and overall excellent herd management. Compared with previously cited Bulgarian studies, a substantial improvement in this trait is evident.

According to Kusaka et al. (2023), advances in genetics and nutritional management have led not only to increased milk yields but also to faster growth rates and greater body weight at first calving. As a result, a decreasing trend in AFC is being reported in many countries for modern Holstein heifers.

The average milk yield per standard lactation in the studied farm is significantly higher than the national average reported by the Bulgarian Executive Agency for Selection and Reproduction in Animal Breeding (Yordanov et al., 2017), which is 5,300–5,600 kg for Black-and-White (Holstein-type) cows, with 3.6–3.8% fat content and 3.2–3.3% protein. These results further highlight the high productivity and biological efficiency of the herd under investigation.

Fig. 1 presents the distribution of cows, expressed as a percentage of the total herd, according to AFC

in days. The lowest proportion of cows (1.18%) had an AFC below 660 days (up to 22 months), followed by those with an AFC over 810 days (more than 27 months) at 3.06%. The highest percentage of cows (60.0%) calved for the first time between 660 and 719 days of age (23–24 months), followed by those with an AFC of 720 to 779 days (24–26 months), accounting for 30.35%. In total, it can be stated that the majority of cows in the herd at the time of the study – 90.35% – had their first calving between 22 and 26 months of age. A total of 8.47% of the animals calved for the first time at over 780 days of age, or more than 26 months.

Gergovska (2011) reported that in approximately 34% of Black-and-White cows, first calving occurred relatively late – at 27–30 months of age with 22.9% calving for the first time at 30–33 months. According to Valchev et al. (2020), among the farms they investigated, the highest proportion of cows first calved at 28–30 months of age (29.53%), while 38.06% calved for the first time at an even more advanced age over 31 months. Only 7.58% of cows calved before 24 months of age.

Te Plate-Church (2002) points out that numerous studies recommend an age at first calving between 20 and 24 months as optimal. However, the ideal age may vary between herds, depending on specific management and environmental conditions.

According to various economic assessments, each additional month of delay beyond 22 months in age at first calving results in a financial loss of approximately \$100 per cow, primarily due to missed production opportunities. Nonetheless, the author emphasizes that body weight at first calving is a more critical factor than age alone.

Meyer et al. (2004) argue that adopting a universal recommendation for a specific AFC may be inappropriate for all cattle breeds or farming systems, as such recommendations might not align with individual farm management goals or production capabilities. Each dairy farm operates under unique

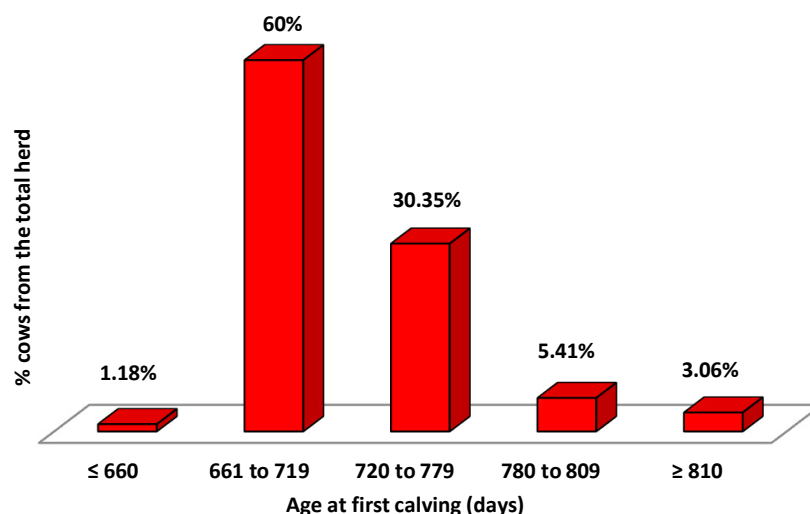


Fig. 1. Percentage of cows depending on age at first calving (days)

management strategies and environmental conditions, making the attainment of an “ideal” AFC and body weight at first calving a complex target. This is further supported by the comparison between our results and those of other national studies, which, although conducted over similar timeframes, reflect differences likely stemming from variations in herd productivity and management practices.

In Bulgaria, like in several other countries, heifers are rarely weighed or subjected to body measurements prior to insemination, which would otherwise serve as a reference for assessing their physical development. Decisions about breeding readiness are most often based on subjective visual assessment. Mourits et al. (2000) report that in the Netherlands, 95% of farmers rely primarily on age to determine the timing of first insemination.

Fig. 2 illustrates the distribution of cows by lactation number at the time of the survey. A strikingly high percentage of cows (39.78%) were in their first lactation. Cows in their second lactation represented 24.47%, slightly more than half of those in their first lactation.

The proportion of cows in their third lactation was 16.47%. Together, cows in their first to third lactation made up the predominant portion of the herd – 80.70%. Cows in their fourth lactation or beyond accounted for 19.30% of the herd. Only a few individuals had reached their sixth or seventh

lactation. These results indicate that a significant proportion of cows entering their first lactation are culled for various reasons before reaching their second or third lactation.

Other authors from different countries have also reported a relatively short productive lifespan for dairy cows. The average productive age of Swedish dairy cows has been estimated at second lactation (Carlén, 2013) to 2.5 lactations (Carlén and Eriksson, 2013). This implies that a cow having her first calf at 30 months of age may not be able to repay the investment in her rearing costs before being culled.

Table 2 presents the results of the analysis of variance regarding the influence of controlled factors on age at first calving.

The year of birth had a statistically significant effect on AFC ($P < 0.05$), whereas the sire did not. As previously noted, the cows were sired by 29 different bulls, with two, three, or more bulls being used in a given year, and some sires being used across multiple years. This suggests that the primary factor affecting AFC is management decisions made over the years, potentially also reflecting the quality of the youngstock and the conception rate at first service in heifers.

Fig. 3 presents mean values of the least squares (LS) obtained from the analysis of variance model for age at first calving by year of birth. It is notable that cows born in 2014 and 2015 had the highest average AFC,

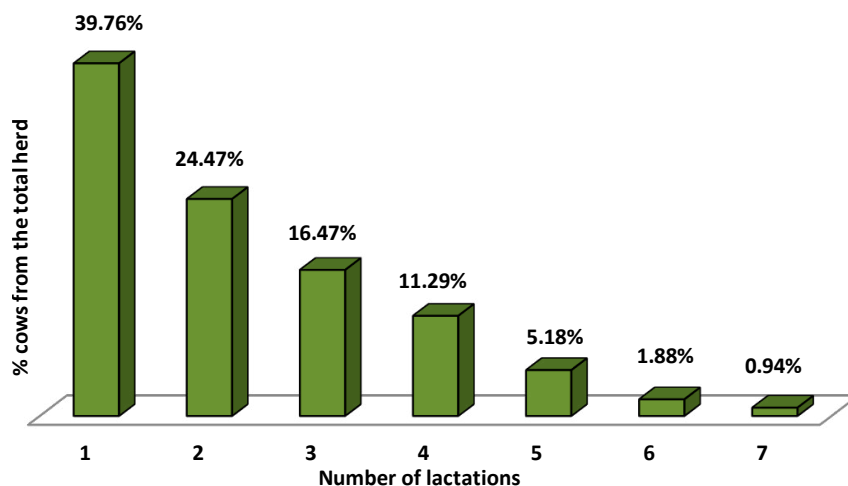


Fig. 2. Percentage of cows by number of lactations

Table . Analysis of variance for the effect of controlled factors on age at first calving

Source of Variation	Degrees of Freedom (df = n - 1)	Age at First Calving	
		MS	F P
Model (overall)	34	2943.36	1.35
Year of birth	6	3327	2.20*
Sire	28	1315	0.87
Error	390	1512	

Note: * – significant at $P < 0.05$; ** – significant at $P < 0.01$; *** – significant at $P < 0.001$; – no significant effect.

747.33 and 730.0 days, respectively, or slightly over 24 months. However, we cannot conclude that heifers in the herd were generally bred at older ages during this period (i.e., 2016–2017), as the sample includes only a small number of cows that had reached their sixth or seventh lactation at the time of the study. Nevertheless, these results may indicate that a higher age at first service, which is typically associated with better physical development, could be a contributing factor to increased longevity in dairy cows. To confirm such a hypothesis, a broader, longitudinal study over multiple years would be required.

Research findings regarding the relationship between AFC and cow longevity are inconsistent. Eastham (2018) suggests that cows calving for the first time between 22 and 26 months are more likely to survive to a second calving. However, this appears to be more reflective of early lactation management practices than of AFC itself. The study by Gävan et al. (2014) reported that survival to second lactation was highest (86.66%) in cows with an AFC of 25–27 months, followed by those calving at 28–30 months (84.21%). In contrast, cows calving at less than 24 months had significantly lower survival rates (71.40%).

Among cows born in 2016, a markedly lower average AFC of approximately 23 months was observed, with a gradual increase in AFC noted

among animals born in subsequent years, reaching an average of 24 months for those born in 2020 and 2021. The data presented for 2022 is incomplete and includes only those animals that had calved by the time of analysis; the remaining animals born that year are expected to have their first calving by the end of 2024.

The above findings are further supported by the data presented in Table 3, which shows the distribution of cows according to age at first calving by their current lactation number at the time of examination. The highest mean AFC was observed in cows that had reached their seventh lactation at 743 days, or nearly 25 months. Conversely, the lowest mean AFC was found in cows at their fourth to sixth lactation, ranging from 697 to 707 days, or slightly over 23 months. However, the range of variation (min-max) reveals that most lactation groups include animals with both low AFC values down to 21.8 months and high AFC values up to 29.1 months. The only exception is the group of cows at their seventh lactation, all of which exhibited AFC values strictly within the 24 to 25 months range.

The variability observed in the other groups, particularly those in their first lactation, indicates that in the farm, insemination of heifers generally begins after they reach one year of age, provided they have attained the required physical development. Delayed

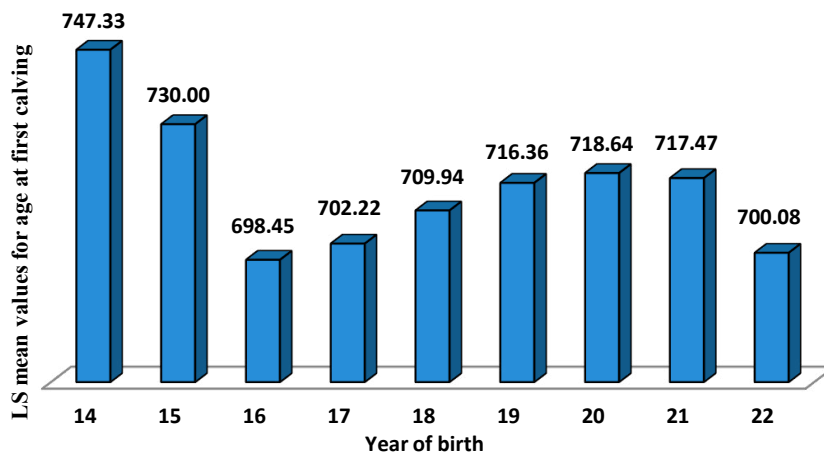


Fig. 3. Means of least squares for age at first calving (days) by year of birth of the cows

Table 3. Mean values and variation in age at first calving of cows according to the number of completed lactations

Number of Lactations	Number	Age at First Calving (Days)			
		$\bar{x} \pm \text{Se}$	SD	min	max
First	169	719.75 ± 3.05	39.68	663	872
Second	104	721.60 ± 4.09	41.74	655	872
Third	70	712.43 ± 4.47	37.37	656	808
Fourth	48	707.21 ± 5.92	41.05	655	804
Fifth	22	697.59 ± 5.41	25.49	674	775
Sixth	8	699.13 ± 6.65	18.81	681	736
Seventh	4	743.00 ± 7.25	14.49	730	757

or omitted insemination due to underdevelopment can lead to breeding at older ages.

Age at first calving is a function of the age at first insemination combined with the reproductive efficiency of the animal. The decision of when to initiate insemination is primarily a management decision. It is generally based on the heifer's age but is also influenced by nutrition and health status. Poor growth during the rearing period, caused by undernutrition and/or disease, has been associated with delayed first insemination and first calving (Brickell et al., 2009; Johnson et al., 2011). Reduced fertility can lead to substantial variation in AFC, often resulting in significant differences between the target and the actual AFC achieved.

Table 4 presents the average milk yield and its variation for all normal lactations (from first to seventh) of all cows present at the time of the survey. The mean milk yield for all first lactations is high – 11 523.28 kg – with some individual yields reaching up to 22 344.36 kg. Average milk yield gradually increases to a peak at the third lactation – 12 562.90 kg – and remains above 12 000 kg through the sixth lactation. Although few in number, cows reaching the seventh lactation have an average yield of 10 550.45 kg per lactation. Maximum yields exceeding 22 000 kg per lactation were recorded in cows from first to third lactations. After the third lactation, maximum

values noticeably decline to below 20 000 kg.

As observed, in addition to milk yield, the number of cows reaching later lactations significantly decreases after the third lactation. This suggests that, beyond the natural decline in milk production with advancing age (or lactation number), high-producing animals in early lactations may have been culled for various reasons. All factors included in the analysis of variance model have a significant effect on milk yield during the cows' first lactation, as shown in Table 5. The most significant factors are the sire of the cow ($P < 0.001$) and the lactation number reached ($P < 0.001$), followed by the year of birth ($P < 0.01$), with age at first calving AFC having the least, yet still significant, effect ($P < 0.05$).

Pytlewski and Antkowiak (2021) also found a significant effect ($P \leq 0.01$) of age at first calving on most of the analyzed first-lactation milk yield traits in Jersey cows.

Year of birth and the lactation number reached are largely interrelated factors. As can be seen from the presented means of least squares (LS means) for the first-lactation milk yield, the milk productivity of first-lactation cows is lowest among those born in the earlier years 2014 and 2015 (just over 8700 kg) and gradually increases exceeding 11 000 kg from 2017 onwards (Fig. 4).

A similar trend was observed regarding the average

Table 4. Mean values and variation in milk yield per consecutive lactation of the cows included in the study

Number of Lactations	Number	Milk Yield for Standard Lactation (from 240 to 305 days)			
		$\bar{x} \pm \text{Se}$	SD	min	max
First	329	11 495.21 \pm 157.98	2865.43	5457.93	22 344.36
Second	226	12 061.53 \pm 203.83	3064.21	5143.19	22 284.67
Third	113	12 561.90 \pm 269.91	2869.20	4443.30	22 690.22
Fourth	53	12 071.04 \pm 288.28	2098.67	7221.18	16 939.40
Fifth	22	12 367.99 \pm 624.00	2926.83	7422.25	18 427.90
Sixth	7	12 188.31 \pm 1240.74	3282.68	8435.30	17 493.39
Seventh	4	10 550.45 \pm 1109.85	2219.70	7823.33	13 245.66

Table 5. Analysis of variance for the effect of controlled factors on age at first calving

Source of Variation	Degrees of Freedom (n – 1)	Milk yield in first lactation	
		MS	F P
Model (overall)	41	19 778 904	3.02***
Year of birth	5	22 656 673	3.45**
Age at first calving	4	35 078 928	3.22*
Number of lactations	6	47 964 386	7.31***
Sire	25	17 722 585	2.70***
Error	287	6 558 112	

Note: * – significant at $P < 0.05$; ** – significant at $P < 0.01$; *** – significant at $P < 0.001$; – no significant effect.

milk yield during the first lactation of cows that reached later successive lactations (Fig. 5).

The lowest average first-lactation milk yields were recorded in cows that reached the seventh and sixth lactations, amounting to 8772.56 kg and 9553.0 kg, respectively. These were cows born in 2014–2015.

In our view, high-yielding heifers are often subjected to more intensive exploitation and experience higher levels of stress, as well as an increased risk of metabolic and reproductive disorders. As a result, they are more frequently culled at an earlier stage, while the herd tends to retain more resilient and better-adapted animals, which are often characterized by more moderate levels of productivity.

The highest average milk yield during the first lactation was observed in the youngest cows currently in their first lactation – 12 045.05 kg. This indicates not only good feeding and management practices implemented on the farm during the study period but also a well-executed selection strategy focused on productivity. In recent years, an increase has also been reported in the first-lactation milk yield of the daughters of the sires used.

Table 6 presents the LS mean values for first-lactation milk yield in cows, depending on their age

at first calving. As shown in Table 3, AFC within the same birth year and successive lactation number ranged from under 22 months to over 25 months. This provides a good basis for evaluating the effect of AFC on the first-lactation milk yield independently of the other two factors.

The lowest first lactation milk yield was recorded in cows with an AFC of less than 659 days (under 22 months), as shown in Table 6. These cows also had the lowest maximum milk yield – 13 495.14 kg. In cows with an AFC between 660 and 809 days (over 22 to 27 months), the first-lactation milk yield was similar – slightly above 11 000 kg – and some of them reached maximum yields of over 20 000 kg. The highest average first-lactation milk yield was observed in cows with an AFC above 27 months – 12 025.54 kg, although this group included a relatively small number of animals.

Pirlo et al. (2000) reported that reducing the age at first calving has a negative impact on both milk yield and milk fat percentage during the first lactation. However, the main reason farmers are reluctant to reduce AFC is the widespread belief that early calving negatively affects productivity and longevity.

The results of Sawa et al. (2019) indicate that

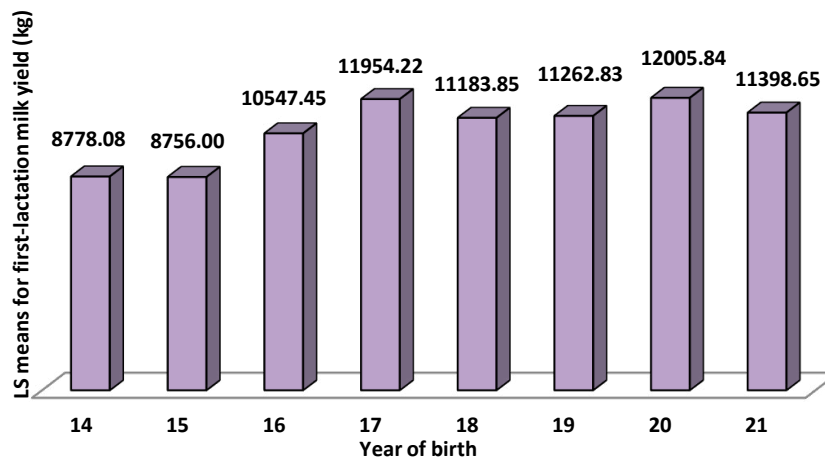


Fig. 4. Means of least squares for first lactation milk yield by year of birth

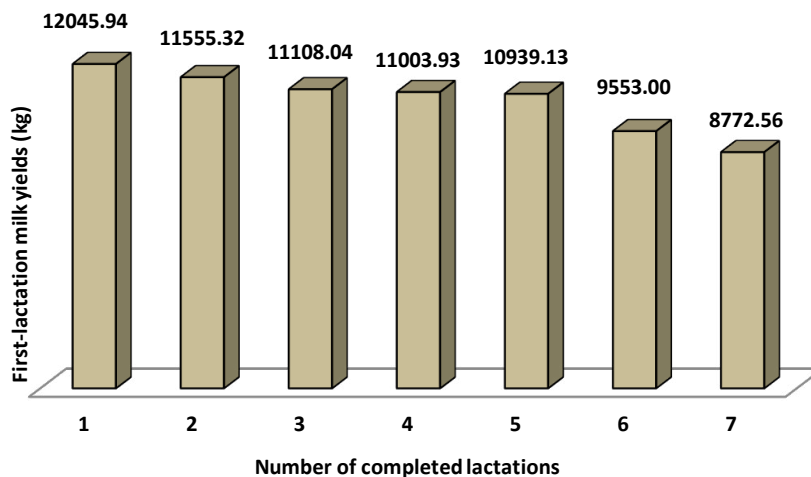


Fig. 5. First lactation milk yield by number of completed lactations

Table 6. Average values and variation of milk yield in first lactation depending on the AFC

Age at First Calving (AFC)	Number	Milk Yield in First Lactation (240 to 305 days)			
		$\bar{x} \pm \text{Se}$	SD	min	max
Up to 650	7	10 330.16 \pm 1239.94	2772.58	7339.31	13 495.14
From 660 to 719	197	11 635.94 \pm 197.03	2785.41	5457.93	21 159.91
From 720 to 779	98	11 373.02 \pm 290.41	2874.83	5875.68	22 344.46
From 780 to 809	18	11 795.75 \pm 753.85	3198.31	8226.06	21 170.49
Over 81	9	12 025.54 \pm 1519.14	4296.77	8267.70	21 333.87

heifers inseminated by 470 days of age have the lowest productivity, while those first inseminated at 711–720 days show the highest milk yield. Further increases in AFC are associated with significant milk losses for producers. According to Elahi Torshizi (2016), if AFC is less than 22 months, milk yield losses during the 305-day first lactation can amount to 590–800 kg.

According to Costigan et al. (2025), when heifers have an AFC between 738 and 768 days and a body weight at first calving between 516 and 550 kg, the productive parameters are optimal.

According to data from Pytlewski and Antkowiak (2021), the highest average daily milk yield (21.9 kg) was recorded in cows calving between 26–28 months of age, while the lowest was observed in cows that calved at the youngest ages.

The study by Mohd et al. (2013) point out that earlier artificial insemination of heifers, even when their development is adequate, results in lower milk yield, especially during the first lactation. On the other hand, Eastham et al. (2018) report that in Holstein cows, a lower AFC (21 months) improves udder health and reproductive indices while also extending the productive lifespan of the animals. According to Kusaka et al. (2023), an AFC of 22.5 months may be associated with better cow survivability and higher lifetime productivity compared with cows with higher AFC, without compromising reproductive performance.

Steele (2020) concludes that most studies suggest that the optimal AFC is between 22 and 25 months. Moreover, both low and high AFC appear to have adverse effects on one or more of the productive or functional traits in dairy cows.

Conclusion

The average age at first calving of cows in the studied farm falls within the optimal and recommended range – 716.26 days (23.88 months) – accompanied by a high average first-lactation milk yield of 11 495.21 kg. A considerable variation in AFC was observed across different birth years and corresponding successive lactations.

Only 1.18% of the cows had an AFC below 660 days (under 22 months). The majority of the cows in the herd at the time of the study – 90.35% – calved for the first time between 22 and 26 months of age. Only 8.47% had their first calving at over 780 days, or more than 26 months of age.

The variation in AFC by successive lactation indicates that insemination of heifers on this farm generally starts after 12 months of age. Failure to conceive or postponed insemination due to insufficient development results in later breeding.

The lowest first-lactation milk yield was recorded in cows with an AFC below 659 days (under 22 months). The highest average first-lactation milk yield was observed in cows with an AFC over 27 months – 12 025.54 kg – although this group was small in number. Cows with an AFC between 660 and 809 days (22 to 27 months) showed similar milk yields, slightly over 11 000 kg, and some of them reached maximum yields exceeding 20 000 kg.

These results indicate that a very low AFC (under 22 months) has a negative impact on first-lactation productivity. Allowing an additional one or two months before first calving (resulting in AFC above 22 months) appears to support better body development in heifers, leading to improved milk production during their first lactation.

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Correlation and Regression Analysis of Cadmium, Lead, Concentrations in Agricultural Soils and Feed Rations of Different Types of Cow Feeding for Prediction and Assessment of Environmental Safety Risk

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Keywords: cattle, heavy metal, agroecosystem, pollutant.

Abstract. Cadmium (Cd) and lead (Pb) as dangerous ecotoxicants pose a significant danger to agroecosystems and all living organisms. They enter the environment due to the action of anthropogenic-technogenic, military factors, global climate change, etc. Data analysis was carried out based on the results of a scientific and economic experiment conducted on dairy cows with different types of feeding in the Forest-Steppe soil-climatic zone of Ukraine. Cows were selected by the method of analogues for live weight and productivity with the third lactation period. The rations included feeds with an excess of Cd and Pb. The ecotoxicity of pollutants affected their transition from soil to plants (feed). The purpose of the research is to analyze the correlational relationship, construct regression equations between the concentration of heavy metals (HM) Cd and Pb in soil samples and feeds of different types of dairy cow diets, which will allow predicting the transfer of pollutants from soil to feed using environmentally safe milk production technologies, carry out environmental monitoring and assess environmental risks on cattle farms in a timely manner. Correlation analysis using the parametric Pearson correlation coefficient and linear regression was performed using the STATISTICA program. In all cases, the analysis established a positive close relationship between the content of heavy metals in soil samples of agroecosystems and feed rations of different types of feeding $r > 0.7$, which indicates a strong correlation dependence and a high predictability of one variable for another. The correlation coefficient for cadmium concentration ranged from 0.799 to 0.971, for lead from 0.790 to 0.869. The highest correlation was established for the content of Cd, where cows were fed a silage-hay-concentrate type ration $r = 0.971$ (very strong correlation). The content of Pb showed a very strong correlation ($r = 0.869$) in a silage-hay ration group. The most acceptable models were for the concentration of Cd in cows with silage-root crops, silage-haylage, and silage-hay-concentrate feeding types, and for the content of Pb in animals with silage-hay and silage-haylage feeding types. A positive close relationship was established between the concentration of Cd and Pb in the soils of agroecosystems and the diets of dairy cows with silage-root crops, silage-hay, silage-haylage, and silage-hay-concentrate feeding type. The best models for predicting Cd in cows on silage-root crops, silage-haylage and silage-hay-concentrate feeding types; Pb \rightarrow silage-hay and silage-haylage.

Introduction

Anthropogenic, technogenic, and military impact on the biosphere leads to contamination of agroecosystems with toxic heavy metals (HM) such as cadmium, lead, copper, zinc, chromium, mercury, nickel, etc., which requires constant monitoring, prevention of spread to large territories, solving the problem of accumulation in soil, water, feed, animal bodies, livestock products, organic waste (Sachko et al., 2016; Saurabh et al., 2023).

Some research studies on soil pollution have been published. In a study by Sazal et al. (2021), 54 dangerous toxicants were found. The researchers selected 40 soil samples around five brick kilns. Multivariate statistics were used to process the results

of the study. Researchers claim that in addition to burning coal in kilns, soil pollution has a negative impact on agriculture. Monitoring environmental pollution with heavy metals is a complex process. To solve the relevant problems, researchers set the task of developing mathematical models for predicting pollution of ecosystems with heavy metals, tested in scientific experiments. Two- and multivariate correlation analysis between heavy metals in different components of the biosphere were used during the statistical analysis of the obtained research results with the construction of appropriate regression equations to predict the level of pollution (Ashraf et al., 2023). Kuramshina et al. (2014) studied the migration of HM from the soil to plants (feed) and the body of animals in the zone of influence of oil and ore deposits with the aim of biologically indicating the

state of the ecosystem, assessing the environmental safety of livestock production in various agricultural areas. The concentrations of elements in the soil and feed were determined, and the coefficients of the transition of HM from feed to the body of animals were calculated. The conclusions made by the researchers are not complicated, but extremely important from a practical point of view: the more elements entered the environment, polluting agroecosystems and plants (feed), the more they were consumed by animals. Sachko et al. (2016) also concluded that there is a relationship between the level of HM in the soil and agricultural crops.

Mingtao et al. (2021) studied the correlation between the content of heavy metals in soil and plants grown to assess the risk of contamination of agricultural crops and predict the danger of harmful effects of toxic metals, especially mercury and cadmium on the human body. Researchers mostly paid attention to the contamination of soil and plants with HM, but did not pay attention to the relationship between them. The results showed that the studied toxic metals in the soil had the greatest impact on the level of zinc in plants, while Pb and Cr had a synergistic effect on the absorption of Zn by plants. The experiments prove the importance of correlation analysis for controlling the risk of contamination, which was not taken into account before.

In our previous studies, we analyzed the correlation between the content of HM in feed rations and organic waste with the construction of appropriate regression equations for assessing the environmental risk of contamination of agricultural lands, predicting the transfer of toxicants from feed to organic waste and organic fertilizers applied to fields for agricultural crops, in order to carry out timely and effective monitoring and avoid contamination of agroecosystems where plants are grown and used as feed for dairy cows (Portyannik, 2024a). There are many examples of the use of correlation and regression analysis both in agriculture and other industries related to environmental pollution in different countries of the world, but the study of the correlation between the content of toxic metals in soil samples and feeds of different types of dairy cow diets with the construction of appropriate regression equations was done by us for the first time and is a relevant study that will facilitate the prediction of the transfer of HM from soil to plants. Cow ration feed in the conditions of environmentally safe milk production technology, both today and in the post-war period, on the basis of the correlation relationship and regression equations were verified in a scientific experiment.

The study of the correlation between the content of toxic metals cadmium and lead in soil samples and feed rations of different types of cow feeding is a convenient and reliable indicator of the forecast of environmental safety in agricultural enterprises.

The use of proven regression equations complements the existing tools for environmental monitoring of agroecosystems.

The purpose of the research is to analyze the correlational relationship, construct regression equations between the concentration of heavy metals Cd and Pb in soil samples and feed of different types of dairy cow rations, which will allow predicting the transfer of pollutants from soil to feed in the conditions of environmentally safe milk production technology, and carry out environmental monitoring and timely assessment of environmental risks on cattle farms.

Materials and Methods

An experiment on dairy cows of Ukrainian black and red-motley dairy breeds for the production of environmentally safe milk was conducted in farms of the Forest-Steppe soil-climatic zone of Ukraine. For the experiments, 126 cows with silage-hay-concentrate feeding type, 63 with silage-hay, 36 with silage-root and 195 with silage-hay feeding type were selected. Cows of all groups were fed feeds containing heavy metals Cd and Pb. The average live weight of cows was 500–545 kg. The average daily milk yield was 14.0–14.8 kg, which is an average of 4270–4514 kg of milk per lactation. The duration of the comparative period was 42 days. Cows selected by the method of analogues for live weight and productivity in their third lactation period were in the same conditions of feeding and were kept in the production conditions of experimental agricultural enterprises with observance of milk production technology. All manipulations with animals were carried out in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (Strasbourg, 1986). Animals of all experimental groups were fed feeds with an increased content of heavy metals Cd and Pb as part of the diets of four types of feeding: silage-root, silage-hay, silage-hay, silage-hay-concentrate. The experiment lasted 120 days.

Before the beginning of the experiment, average soil samples of agricultural lands were taken at a depth of 25 cm, where plants were grown and used to produce feed, included in the diets of different types of feeding for the production of cow's milk. The root system of plants, which absorbs heavy metals from the soil, is located at a depth of 25 cm. Samples were taken before harvesting in the spring on land plots where the corresponding plants from which feed was produced were grown. In total, 25 point samples were taken from one plot with an average area of 5 hectares. The field was divided into equal squares of 2.5 hectares. A soil auger and a shovel were used. All point samples were placed in a clean bucket and mixed thoroughly. After that, 1 kg of soil was taken, as an average sample. It was dried at room temperature without heating. Plant residues and stones were removed. The average sample was packed in a paper

bag on which the experimental farm, field number, area, sampling depth, crop, and date were indicated.

At the beginning of the experiment, average samples of the following feeds were selected: cereal-legume hay, wheat straw, corn silage, alfalfa haylage, fodder beet, corn husks in silage-horseradish ration; alfalfa hay, wheat straw, corn silage, alfalfa haylage, fodder beet, barley husks in silage-hay; alfalfa hay, cereal-legume hay, corn silage, alfalfa haylage, pea husks in silage-hay; alfalfa hay, cereal-legume hay, corn silage, alfalfa haylage, corn husks, pea husks in silage-hay-concentrate. Feed samples were selected from the entire batch so that one average sample was representative, reflecting the quality of the entire batch. Clean tools, samplers, and shovels were used. Several spot samples were taken from different places and combined into one average sample weighing 1 kg. The sample was placed in a clean dry bag. An appropriate sampling report was drawn up. The litter was taken from different layers: top, middle, bottom. Hay and straw samples were taken with a special probe from different bales of at least 10% of the batch. Samples from different bales were combined and thoroughly mixed to obtain an average sample. Silage and haylage samples were taken from different depths of the trench, avoiding surface or spoiled layers. They were stored in a sealed bag to avoid moisture loss. The accompanying document indicated the name of the feed, manufacturer, batch number, date of sampling, etc.

Analysis of soil and feed samples for heavy metal content was carried out by atomic absorption spectrophotometry (AAS-30 spectrophotometer). To calculate the correlation dependence, only mobile forms of pollutants in the soil were taken into account.

Data analysis was carried out taking into account the features of the results obtained in the study: sample size and type of data distribution, and the nature of dispersions. Correlation dependence was considered statistically significant at $P < 0.05$. Correlation analysis was carried out using the parametric Pearson correlation coefficient taking into account the Kolmogorov-Smirnov and Lilliefors tests for normality and the Shapiro-Wilk's W test. For this purpose, a sample of six ($n = 6$) results of laboratory chemical analysis of average soil samples for the content of mobile forms of heavy metals in mg/kg and six ($n = 6$) average feed samples with the content of heavy metals in mg/kg, which were included in the corresponding basic feeding rations of different types of the first control groups, was taken. For the method of modeling the dependence between the variable U (concentration of heavy metals in feed rations) and the vector variable X (concentration of heavy metals in soils of agroecosystems), the construction of linear regression equations was used. The nature of the relationship between the indicators was checked by constructing scatterplots. The analysis of the residuals was checked for compliance with the law

of normal distribution (Gaussian). The assessment of the acceptability of the model as a whole by its level of probability was assessed using the ANOVA method. The quality of the calculated regression was checked using the coefficient of determination R^2 . It was considered that if R^2 is less than 0.3 (less than 30% – very low level), the model will most likely not work reliably. The calculation was performed in the STATISTICA software package version 10.0. The interpretation of correlation data was carried out on the scale (Gorkavyi, 2019) where $r < 0.3$ – no relationship, the variation of one variable almost does not explain the other, often has no practical significance; $r = 0.3-0.5$ – the relationship is weak, weak correlation; $r = 0.5-0.7$ – the relationship is average (moderate), a significant proportion of the variation is explained; $r > 0.7$ – the relationship is close, strong correlation, high predictability of one variable by another.

The choice of linear regression is due to the logical method, a linear relationship is expected between the variables according to physicochemical laws. The more mobile forms of heavy metals are contained in the soil, the greater their amount will be absorbed by the root system of plants, the greater the amount of pollutants will pass into the feed. The theory does not assume curvilinearity, so there is no need to complicate the model. Graphical analysis of the data showed an approximate linearity of the relationship between the variables. Linear regression is easier to interpret and more stable for small samples as in our case. From the point of view of practical interpretation, the coefficient in the linear model has a simple explanation. When X increases by 1 unit, Y changes on average by β units.

Results

Exceeding the maximum permissible concentration of mobile forms of cadmium and lead in the soils of agricultural lands of agricultural enterprises caused their transfer to plants, which were used as feed for dairy cows. The probability according to the Shapiro-Wilk's W test for the concentration of cadmium in the soils of agroecosystems is $P = 0.601$, $P = 0.993$, $P = 0.993$, $P = 0.399$, for the content of the pollutant in the feeds of the main diets $P = 0.103$, $P = 0.654$, $P = 0.782$, $P = 0.871$, respectively, which is significantly greater than 0.05 ($P > 0.05$). The Kolmogorov-Smirnov test in all cases is also greater than 0.05 and is $P > 0.20$. The Lilliefors test for normality is also higher than 0.05, as is the Kolmogorov-Smirnov test ($P > 0.20$), except for the feeds of the silage-root crops diet the probability ($P < 0.20$). For lead concentration in agricultural soils, the probability according to the Shapiro-Wilk's W test is $P = 0.315$, $P = 0.953$, $P = 0.855$, $P = 0.286$, for the pollutant content in the main rations, $P = 0.163$, $P = 0.215$, $P = 0.564$, $P = 0.161$, which is also significantly higher than 0.05 ($P > 0.05$). For

cadmium concentration, the Kolmogorov-Smirnov test is also in all cases higher than 0.05 and is $P > 0.20$. The Lilliefors test for normality is higher than 0.05, as is the Kolmogorov-Smirnov test ($P > 0.20$). Only in the soil of agricultural lands where cows were kept on cattle farms with a silage-root crops type of feeding, the probability according to the Lilliefors test for normality is $P < 0.20$, and in the soil and feed of the diet where animals were kept with a silage-haylage-concentrate type of feeding, the probability is also $P < 0.20$ and $P < 0.15$, respectively. For all tests conducted, the error is significantly greater than 0.05 ($P > 0.05$), the hypothesis is correct, and the distribution of data that makes up the corresponding samples does not differ statistically from normal. Such a distribution can be considered normal, one that corresponds to the law

of normal distribution (Gaussian). Therefore, for the accuracy of correlation and regression analysis, we choose parametric methods. Correlation analysis is performed by the Pearson method, not Spearman, and regression analysis is performed without logarithmic transformation of data.

Table 1 shows the concentration of cadmium and lead in the selected soil samples and feed, as well as the calculated Pearson correlation coefficients between the content of toxic elements under different types of feeding of dairy cows.

The correlation coefficient between the content of heavy metals cadmium and lead in the soils of agroecosystems and feed rations of different types of feeding of dairy cows in all analyzed cases is positive, the relationship is close $r > 0.7$, i.e., there is a strong

Table 1. Content of cadmium and lead in soils of agroecosystems and feeds of basic diets of different types of cow feeding and Pearson correlation coefficient, mg/kg $n = 6$

Feeding type	Heavy Metal Concentration				Correlation Coefficient	
	Soil		Feed			
	Cadmium	Plumbum	Cadmium	Plumbum	Cadmium	Plumbum
Silage-root crops	0.58	17.52	0.96	28.59	0.902	0.810
	0.5	12.06	0.71	13.62		
	0.43	14.62	0.69	13.51		
	0.42	11.41	0.75	16.78		
	0.37	11.92	0.64	12.22		
	0.56	16.05	0.96	20.51		
Silage-hay	0.55	16.97	0.51	12.5	0.799	0.869
	0.47	14.92	0.39	9.13		
	0.53	13.44	0.45	9.54		
	0.62	18.27	0.75	17.21		
	0.5	16.13	0.57	11.22		
	0.58	14.75	0.54	11.6		
Silage-haylage	0.34	11.07	0.66	18.5	0.859	0.850
	0.27	9.64	0.48	12.5		
	0.39	14.12	0.54	15.5		
	0.44	12.03	0.63	16.51		
	0.34	11.07	0.66	18.5		
	0.27	9.64	0.48	12.5		
	0.39	14.12	0.54	15.5		
	0.44	12.03	0.63	16.51		
Silage-haylage-concentrate	0.51	16.34	0.75	36.51	0.971	0.790
	0.3	10.64	0.42	10.54		
	0.31	13.54	0.48	14.23		
	0.33	11.21	0.54	18.51		
Silage-haylage-concentrate	0.45	11.08	0.69	16.52	0.971	0.790
	0.39	15.07	0.63	20.53		

Note: the probability of correlation $P < 0.05$.

Source: developed by the authors.

correlation and high predictability of one variable for another. The smallest correlation between the indicators where cows were kept with silage-hay type of feeding was established at the level of $r = 0.799$ for cadmium concentration, and at the level of $r = 0.790$ for lead concentration, where cows were kept on a silage-haylage-concentrate ration. The probability of the correlation in all cases is $P < 0.05$ (Figs. 3–4). The highest correlation coefficient was established for the cadmium content, where the animals were fed a silage-haylage-concentrate type diet $r = 0.971$ (very high (strong) correlation) ($P < 0.05$), while for the lead content with silage-hay diet $r = 0.869$ (very high (strong) correlation) ($P < 0.05$). Therefore, the correlation coefficients for cadmium ranged from 0.799 to 0.971, and for lead from 0.790 to 0.869 ($P < 0.05$).

Application of the parametric analysis method in the form of the Pearson correlation coefficient using the STATISTICA program allowed us to calculate and thereby reliably ($P < 0.05$) in all cases establish a close correlation, a strong correlation between the content of toxic HM Cd and Pb in the soils of the Spodar agricultural lands and the feed of the rations of different types of feeding of productive animals, which facilitates the regression analysis.

We checked the nature of the relationship

between the two variables of cadmium and lead concentrations. We constructed scatter diagrams (Figs. 1–2). The location of the points on the diagram had an appropriate dispersion across the canvas, there were no clusters or shifts in any one direction, etc. (Figs. 1–2).

We built a histogram of residuals and checked for compliance with the normal distribution law (Figs. 3–4). The residuals were scattered more or less symmetrically (Figs. 3–4). The normality hypothesis was not rejected.

We looked at the normal-probability plot of residuals. No systematic deviations of the actual data from the theoretical normal line were observed. The residuals were distributed normally. We checked the presence or absence of the dependence of the residuals on the predicted values. No specific systematic direction of movement of the points was observed. Otherwise, we had to state the corresponding dependence. The points did not have a systematic arrangement. They were arranged randomly on the diagram canvas, which proves the independence of the residuals from the predicted values (Figs. 3–4). All conditions are met, and the analysis of residuals shows that our model is quite good for this parameter.

We assessed the acceptability of the model as a whole by the ANOVA parameter and the level

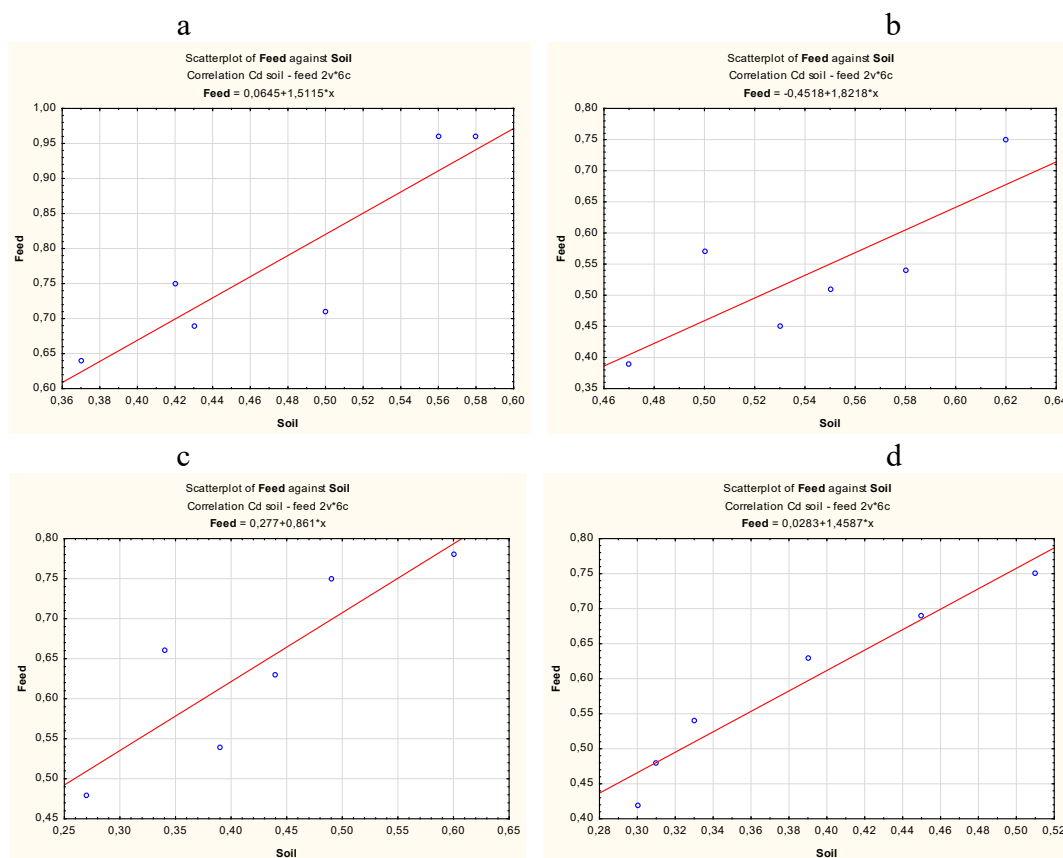


Fig. 1. Scatter diagram for data on Cd content (mg/kg) in agricultural soils and feeds of the main diet with the corresponding regression equation

Types of feeding: a – silage-root crops $y = 0.0645 + 1.5115 \times x$; b – silage-hay $y = -0.4518 + 1.8218 \times x$; c – silage-haylage $y = 0.277 + 0.861 \times x$; d – silage-haylage-concentrate $y = 0.0283 + 1.4587 \times x$.

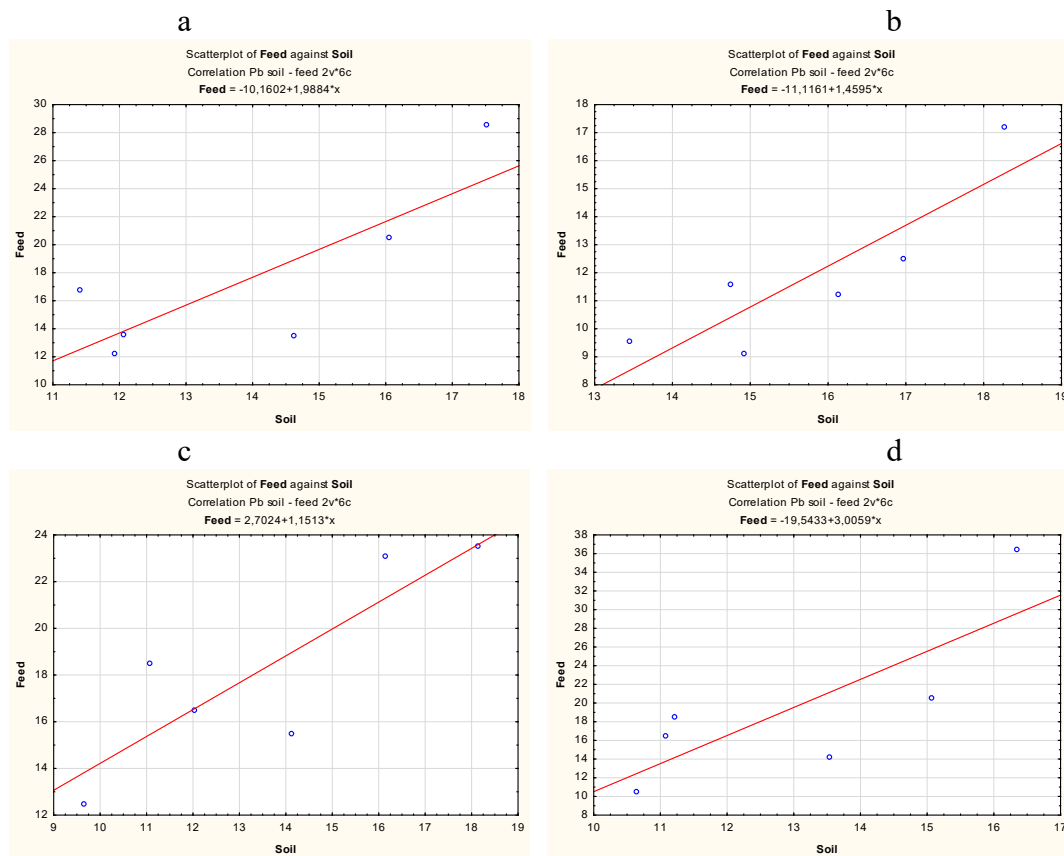


Fig. 2. Scatter diagram for data on Pb content (mg/kg) in agricultural soils and feeds of the main diet with the corresponding regression equation

Types of feeding: a – silage-root crops $y = -10.1602 + 1.9884 \times x$; b – silage-hay $y = -11.1161 + 1.4595 \times x$; c – silage-haylage $y = 2.7024 + 1.1513 \times x$; d – silage-haylage-concentrate $y = -19.5433 + 3.0059 \times x$

of probability. The level of probability for the concentration of cadmium was $P = 0.014$, $P = 0.057$, $P = 0.028$, $P = 0.001$, while for lead it was $P = 0.051$, $P = 0.025$, $P = 0.032$, $P = 0.062$, respectively. The most acceptable model for the concentration of cadmium in animals with silage-root, silage-sage and silage-sage-concentrate feeding type was $P < 0.05$, and for the content of lead, the model in animals with silage-hay and silage-sage feeding type was $P < 0.05$. These models will work much better than the forecast based on average values. All other models do not have a probability level of $P < 0.05$, so the forecast accuracy may be worse.

The coefficient of determination R^2 varies from 0 to 1 and indicates how many factors that affect the response are taken into account in the model. R^2 for Cd concentration was 0.81 (81%) (silage-root crops type of feeding), 0.64 (64%) (silage-hay), 0.74 (74%) (silage-haylage), and 0.94 (94%) (silage-haylage-concentrate). If R^2 is less than 0.3 (less than 30% is a very low level), it will indicate that the model will most likely not work reliably. In our case, there were no such models. Therefore, the probability of predicting the concentration of the pollutant in the feed rations according to the corresponding regression equations is high. R^2 for Pb concentration is 0.66 (66%) for silage-root crops type of feeding, 0.76

(76%) for silage-hay, 0.72 (72%) for silage-haylage, and 0.62 (62%) for silage-haylage-concentrate. For lead content, R^2 less than 0.3 (less than 30% is a very low level) was also not found. The number of factors taken into account when building the model is high. This means that all models will work with sufficient probability. All probability levels that are $P < 0.05$ are acceptable for building the model. In our case, for Cd concentration, $P < 0.014$ (acceptable) for silage-root type of feeding, $P < 0.057$ (unacceptable) for silage-hay type of feeding, $P < 0.028$ (acceptable) for silage-haylage type of feeding, $P < 0.001$ (acceptable) for silage-haylage-concentrate type of feeding. For Pb, the values were $P < 0.051$ (unacceptable), $P < 0.025$ (acceptable), $P < 0.032$ (acceptable), and $P < 0.062$ (unacceptable) for silage-haylage-concentrate type of feeding, respectively.

Discussion

The consequences of heavy metal contamination of the trophic chain are critical. Pollutants, getting into the soil, are actively absorbed by plants, accumulate in root crops, cereals and leafy vegetables. Contaminated feed becomes a source of heavy metal accumulation in animal products, such as meat, milk and eggs, which poses a threat to food security and public health, as noted by Gulich et al. (2025), with

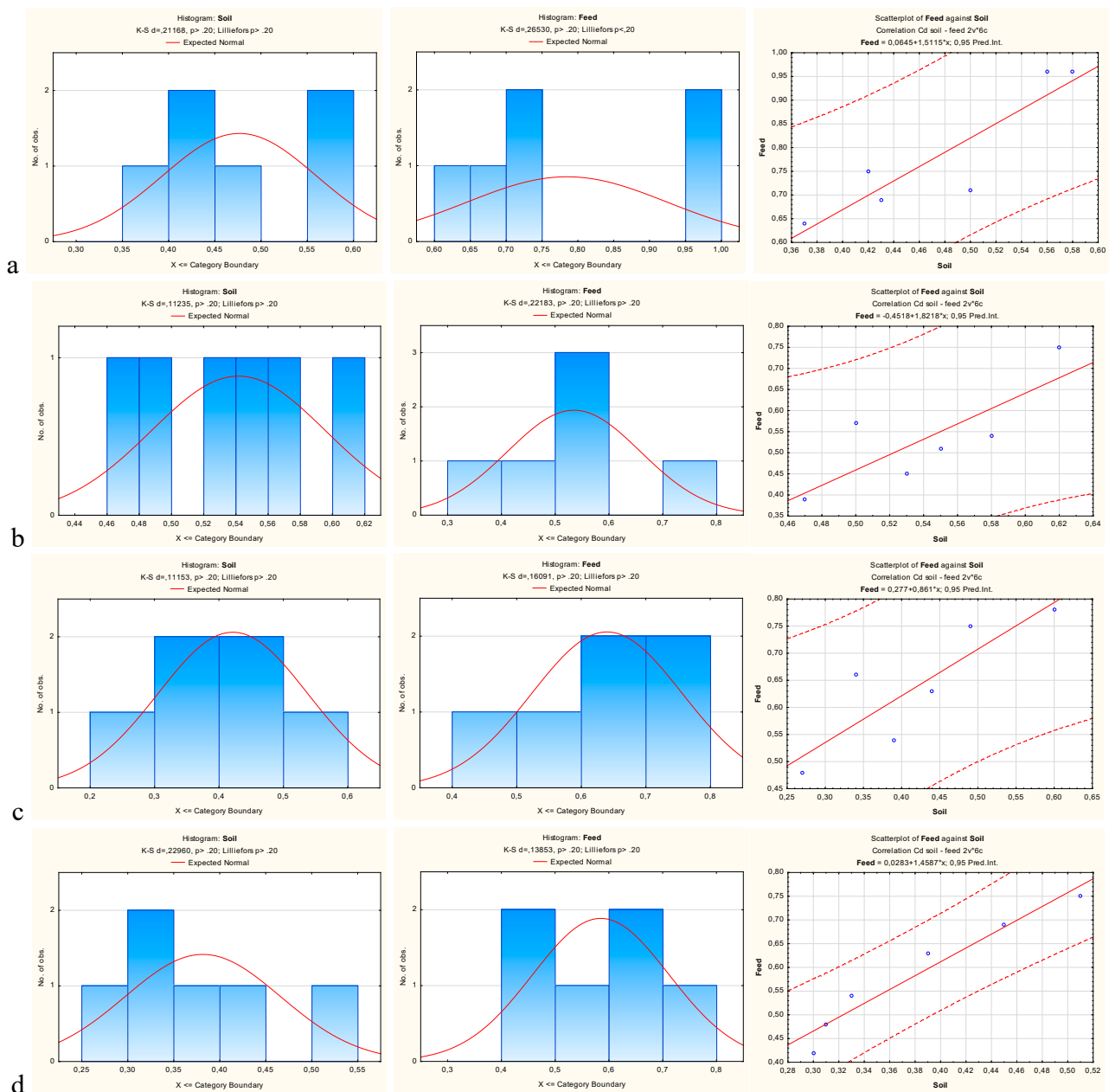


Fig. 3. Analysis of residues by Cd concentration (mg/kg) in soils of agroecosystems and feeds of rations of different types of cow feeding types of feeding: a – silage-root crops; b – silage-hay; c – silage-haylage; d – silage-haylage-concentrate.

which we also agree (Portiannyk et al., 2024b). The study of heavy metals as a factor of environmental pollution during war is extremely important, since they constitute one of the most dangerous groups of pollutants. Military operations significantly increase the anthropogenic load on ecosystems, leading to the accumulation of toxic elements in soil, water and biological objects. In the post-war period, the accumulation of heavy metals in the soils of various regions of Ukraine may significantly exceed safety standards; global warming and climate change also lead to an increase in the concentration of heavy metals in the soils of ecosystems (Xiao et al., 2024; Wrzeczńska et al., 2021). Agricultural lands in the pre-war period were subjected to significant technogenic pollution,

while today's hostilities only increase the pollution with heavy metals. Mining, air defense work, when unmanned aerial vehicles and missiles crash mainly somewhere in the field, rather than over residential buildings, avoiding a threat to human life, ultimately lead to the dispersion and accumulation of mercury, cadmium, lead, copper, zinc, chromium and other HMs in the soil of agricultural lands.

Measuring environmental parameters in the broadest sense – monitoring, sampling, laboratory analysis, etc. – often entails significant challenges. Significant costs, time constraints, inherent uncontrolled conditions of on-site measurements can lead to insufficient and/or low-quality data with a small number of repetitions, which needs to be taken

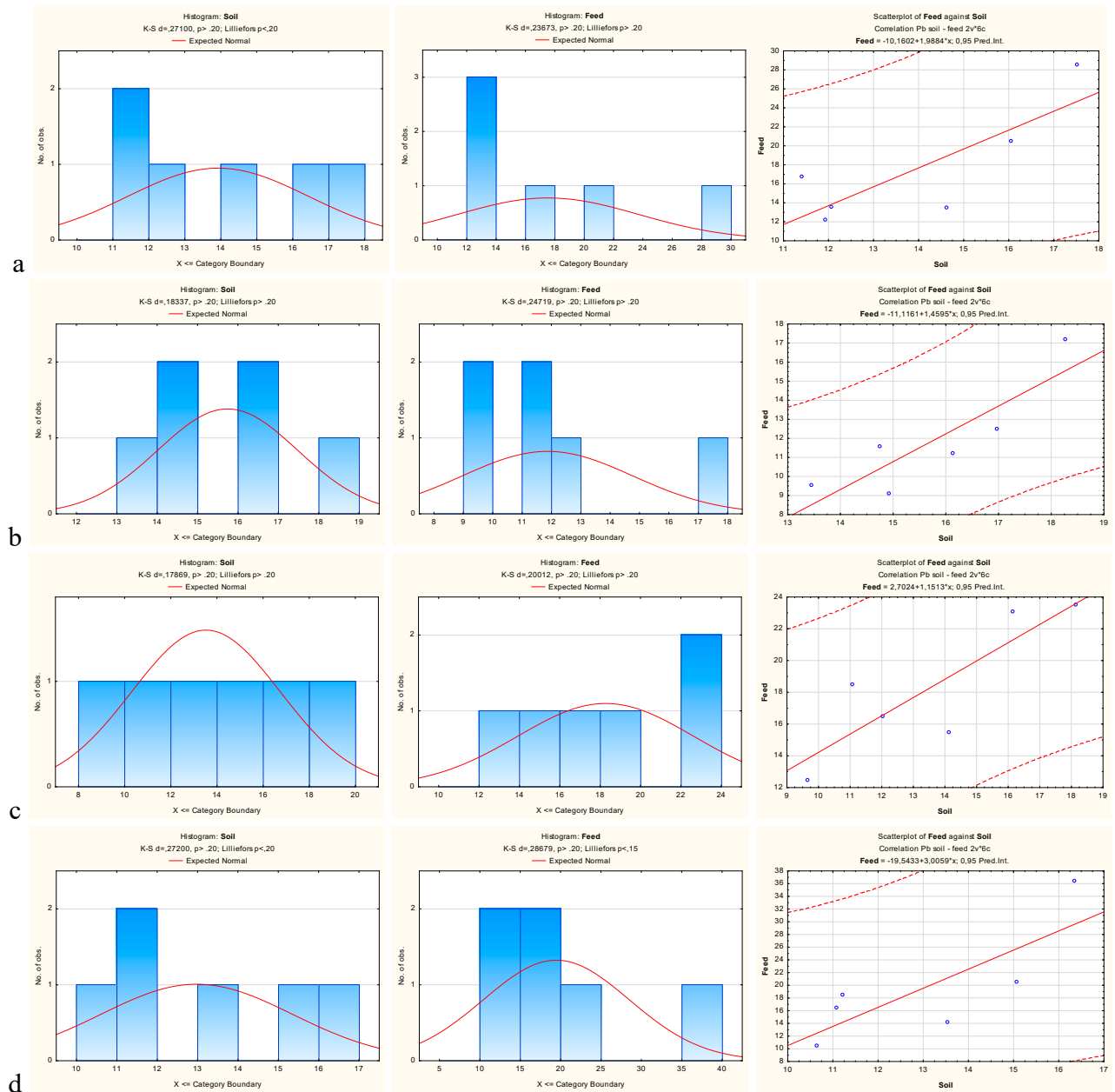


Fig. 4. Analysis of residues by Pb concentration (mg/kg) in soils of agroecosystems and feed ratios of different types of cow feeding

Types of feeding: a – silage-root crops; b – silage-hay; c – silage-haylage; d – silage-haylage-concentrate.

into account by researchers. In general, methods based on correlation and regression aim to go beyond (usually graphical) evidence of a certain relationship between variables and, possibly, to consolidate them, with different goals: from quantifying the degree of the relationship (for example, using the correlation coefficient) to developing a predictive model. A number of methods have been developed for the above-mentioned goals, which, like any statistical model, are based on their own fundamental assumptions, and are now widely used and implemented in most existing data analysis tools. For example, Damien et al. (2025) in their work consider a number of important methodological approaches that researchers need to take into account in order

to develop qualitative models during correlation and regression data analysis. Analyzing a large number of publications related to correlation and regression analysis, researchers come to the conclusion that most environmental scientists face the inherent problem of establishing certain evidence about complex processes, which are often estimated using limited, partial and/or uncertain data. To this end, methods inherited from statistical modeling open up a wide range of possibilities, overcoming the mechanistic description of phenomena when this is practically impossible to implement. Therefore, the introduction of correlation and regression approaches in environmental research is understandable and in many cases fully justified. It is also likely that these approaches have been

facilitated by the democratization of statistical tools and software, which facilitate data processing and analysis. The STATISTICA program we used allows not only performing all statistical and analytical calculations, but also using it for convenience in practice. Of course, it is possible to use other similar programs, which are many. It is also recommended not to violate the assumption of normality for data testing. When using and distributing the model, in order to avoid unnecessary confusion, researchers (Damien et al., 2025) recommend employing the coefficient of determination when the goal is prediction, and correlation when describing the relationships between variables. The conclusion in regression is based on the theoretical properties of the residuals, which should be checked, which we took into account in the process of this analysis.

Researchers widely use methods of correlation and regression analysis of research results. Various computer programs and analysis algorithms are used. Not only the analysis of the strength of the correlation between indicators is important, but also the correct choice of indicators themselves. Correlation analysis has been used by researchers to identify the relationship between HM in marine sediments of Hong Kong (Lili et al., 2015). The Spearman rank correlation was used in the first stage, followed by the Pearson correlation in the second. The bay and harbor (Victoria) were polluted with Pb and Cu, which correlated with Cr, Ni and Zn pollution (Lili et al., 2015). The researchers believe that they were the first to apply such analysis to the study of environmental pollution. In addition, such methods have already been widely used in biology, sociology, computer science, etc. We believe that correlation and regression analysis can be applied not only to marine sediments or any other environmental studies. In our case, the importance of such analysis increases due to the complex, multifaceted processes occurring in agroecosystems, where living organisms, especially dairy cows, play a major role in producing environmentally safe dairy products; meanwhile, the main criteria remain quality and accuracy, which we ensured by incorporating the methodological developments of other scientists as much as possible.

We carried out the entire analysis in stages. First, we established the correspondence of the research results to the law of normal distribution (Gaussian), and then applied the powerful and reliable Kolmogorov-Smirnov and Lilliefors test for normality and Shapiro-Wilk's W test. These tests are effective for small samples, as in our case ($n = 6$). According to all the tests performed, the error $P > 0.05$. The hypothesis is correct, the data distribution does not statistically differ from normal, i.e., it obeys the law of normal distribution (Gaussian). Next, we applied the Pearson parametric method and calculated the correlation coefficients between the content of cadmium and lead in agricultural soils and feed rations of different types

of feeding of productive animals. In all cases, the coefficient is positive $r > 0.7$, which indicates a strong (high) correlation, and the probability of correlation in all cases is $P < 0.05$. The correlation coefficients ranged from 0.799 to 0.971 for Cd, and from 0.790 to 0.869 for Pb ($P < 0.05$). The results of our correlation analysis are consistent with other studies (Sachko et al., 2016), which also established a high correlation at the level of 0.80–0.93 between the content of HM in soil and forages, in particular meadow hay and wheat straw.

Before conducting the regression analysis, we checked the nature of the relationship between the two variables for both cadmium and lead. The constructed scatter plots do not show any clusters in any one direction. Residual analysis is important in regression analysis. Therefore, we constructed a histogram of the residuals and checked for compliance with the normal distribution law. The normality hypothesis was not rejected. The residuals were normally distributed. It is important that there is no dependence of the residuals on the predicted values, so the model is quite good and we can further assess its acceptability as a whole. Taking into account the methodology (Damien et al., 2025), checking the significance of the model is of great importance. We applied the ANOVA parameter. It turned out that the most acceptable model for predicting the transfer of the element from soil to feed in animals with silage-root crops, silage-haylage and silage-haylage-concentrate diets for cadmium concentration was with silage-hay and silage-haylage diets for lead ($P < 0.05$). Other models do not have such a level of probability. Therefore, their application may have a greater error in use. We performed an analysis of R^2 determination, which is recommended (Damien et al., 2025; Boldizar et al., 2017). The biosorption characteristics of Cd and Zn ions from monocomponent aqueous solutions by the macromushroom *Agaricus bisporus* were studied. Initial concentration of metal ions, contact time, initial pH and temperature were the parameters that affected biosorption. Experimental data were analyzed using kinetic models, and various models were calculated in linear and nonlinear (CMA-ES optimization algorithm) regression. Langmuir and Freundlich coefficients were calculated using linear regression for Cd and Zn biosorption on *Agaricus bisporus* macrofungus biomass. Boldizar et al. (2017) provide the corresponding graphs that indicate the R^2 determination index and the corresponding regression equation. In their experiment, R^2 is very high, 97–99%. In our case, R^2 for Cd concentration ranged from 0.64 to 0.94 (64–94%) and Pb from 0.62 to 0.76 (62–76%). However, we do not have models with a very low coefficient of determination R^2 below 30% in our analysis. This means that all models will work with sufficient probability. In terms of probability, the models for cadmium concentration for silage-root crops feeding type ($P < 0.014$), silage-haylage

($P < 0.028$), silage-haylage-concentrate ($P < 0.001$), lead in silage-hay ($P < 0.025$) and silage-haylage ($P < 0.032$) are acceptable.

After constructing the regression equations, we tested them on examples to verify the accuracy and effectiveness of the developed models. Laboratory analysis of selected soil samples from agricultural lands where plants are grown for fodder and bark is used in the rations of different types of feeding of dairy cows established the concentration of cadmium at 0.48 mg/kg; however, the concentration in the feed is unknown. Animals are fed the silage-root crops type of feeding. We substituted this indicator into the program, or the corresponding regression equation $y = 0.0645 + 1.5115 \times x$ and obtained the result $0.790 = 0.0645 + 1.5115 \times 0.48$. The predicted concentration of Cd in the feed of the corresponding type of diet is 0.790 mg/kg. This result is consistent with the actual results of laboratory analysis of the feed given in the original tabular data. We also checked all other forecast models, including Pb. We checked the forecast of the concentration of heavy metal Cd in the feed of the silage-haylage diet. The concentration of the pollutant in soil samples was 0.44 mg/kg ($0.350 = -0.4518 + 1.8218 \times 0.44$). The predicted concentration of Cd in the feed of the ration that will be fed to dairy cows according to the corresponding type of feeding is 0.350 mg/kg. This result is consistent with the actual results of laboratory analysis of feeds given in the original tabular data. Next, we checked the forecast of the concentration of Cd in the feed of the silage-haylage-concentrate type of diet. The concentration of the pollutant in soil samples was 0.52 mg/kg ($0.725 = 0.277 + 0.861 \times 0.52$). The predicted concentration of Cd in the feed of this cow ration was 0.725 mg/kg. This result is consistent with the actual results of laboratory analysis of the feed of the ration given in the original tabular data. We checked the forecast of the concentration of Cd in the feed of the ration of dairy cows with the silage-hay-concentrate type of feeding. The concentration of the pollutant in the soil samples was 0.37 mg/kg ($0.568 = 0.0283 + 1.4587 \times 0.37$). The predicted concentration of Cd in the cow feed was 0.568 mg/kg. This result is consistent with the actual concentration obtained after laboratory analysis of feed, which is given in the original tabular data. Similarly, we checked the values for lead. Chemical analysis of soil samples from agroecosystems where plants were grown for feed included in the diets of various types of feeding of dairy cows, established the concentration of lead at 13.45 mg/kg; however, the concentration in the feed rations is unknown. The animals were fed according to the silage-root crops type of feeding. We substituted this indicator into the program, or the corresponding regression equation $y = -10.1602 + 1.9884 \times x$ and obtained the result $16.584 = -10.1602 + 1.9884 \times 13.45$. The predicted concentration of Pb in the feed of

the diet of the corresponding type was 16.584 mg/kg (the result agrees). We checked the prediction of the concentration of Pb in the feed of the diet of cows with the silage-hay type of feeding. The concentration of the pollutant in the soil samples was 12.32 mg/kg ($6.865 = -11.1161 + 1.4595 \times 12.32$). The predicted concentration of Pb in the feed of the diet of this type was 6.865 mg/kg (the result is consistent). Next, we checked the prediction of the concentration of Pb in the feed of the diet of cows with the silage-haylage feed type. The concentration of the pollutant in the soil samples was 13.67 mg/kg ($18.440 = 2.7024 + 1.1513 \times 13.67$). The predicted concentration of Pb in the feed of the diet of the corresponding type of feed was 18.440 mg/kg (the result is consistent). We checked the prediction of Pb concentration in the feed of the diet of cows with silage-haylage-concentrate type of feeding. The concentration of the pollutant in soil samples was 12.24 mg/kg ($17.249 = -19.5433 + 3.0059 \times 12.24$). The predicted concentration of Pb in the feed of the diet of this type was 17.249 mg/kg and the result is consistent with the actual concentration obtained after laboratory analysis of the feed, which is given in the original tabular data.

We checked all the regression equations we calculated by introducing, as an example, instead of X, the concentration of cadmium or lead in soil samples, conditionally known from laboratory analysis, and obtained the corresponding predicted concentration of the pollutant in the feed of rations of different types of cow feeding. The calculation was carried out in two ways: simple mathematical calculation and using the STATISTICA program, which includes a built-in function for this purpose. All regression equations showed an acceptable result, but it is more necessary to apply those models where high probability is established. In the conditions of our model, only two parameters are set: the first is the concentration of cadmium and lead in soil samples of agroecosystems and the second is the concentration of cadmium and lead in the feed of different animal rations. Model testing using the Kolmogorov-Smirnov and Lilliefors test for normality and Shapiro-Wilk's W test, Pearson parametric correlation analysis, construction of scatter plots, residual analysis, assessment of model acceptability using the ANOVA parameter and the R^2 coefficient of determination allows us to select the most effective and most accurate regression equations for forecasting. After the comprehensive analysis and at the final stage of the corresponding verification, we recommend using the constructed regression equations for the concentration of Cd in the silage-root crops diet $y = 0.0645 + 1.5115 \times x$, silage-hay $y = -0.4518 + 1.8218 \times x$, silage-haylage $y = 0.277 + 0.861 \times x$, silage-haylage-concentrate $y = 0.0283 + 1.4587 \times x$, and the following for the concentration of Pb $y = -10.1602 + 1.9884 \times x$, $y = -11.1161 + 1.4595 \times x$, $y = 2.7024 + 1.1513 \times x$,

$y = -19.5433 + 3.0059 \times x$, respectively, as a tool for environmental monitoring, forecasting (modeling) the transition of toxic HM cadmium and lead in feed rations of various types of feeding of dairy cows on cattle farms.

Multiple linear regression was used (Weibin et al., 2022) in the study of HM pollution in an industrial park. Researchers have

created a method that combines principal component analysis, geodetector, and multiple linear regression of the distance to the sources of pollutant emissions, which affects the level of soil pollution. The main sources of pollution with specific elements were identified by researchers using regression equations. Identification of potential sources of pollution is the basis of environmental risk control. Analysis of sources of HM soil pollution is one of the most important problems in recent years (Yanxue et al., 2017; Xufeng et al., 2020; Geng et al., 2020; Weibin et al., 2022) as well as the problem of producing environmentally safe milk. Milk and dairy products are important in human nutrition. For example, milk from animals fed on feed grown on agricultural land irrigated with wastewater poses a risk for Cd, Cr, Cu, Mn, Ni and Pb (Sena et al., 2023). A study conducted in the Algerian region analyzed 88 raw milk samples obtained from local animal breeds. According to the results of the study, there is a potential risk of HM contamination, especially Pb, for infants due to the consumption of raw milk (Boudebbouz et al., 2023). Another study conducted in Egypt analyzed a total of 75 dairy products, including raw cow milk, raw buffalo milk, condensed milk, infant formula and powdered milk. The results showed that the levels of toxic metals Pb and Cd were higher than the recommended daily intake (RDI) (Sena et al., 2023). In another study comparing heavy metal levels in cow and buffalo milk, the mean concentrations of Pb, Cd, Cu, and Ni in cow milk samples were 0.62 ± 0.25 , 0.25 ± 0.22 , 0.31 ± 0.20 , and 21 ± 2 mg/kg, respectively, while in buffalo milk, they were 0.60 ± 0.3 , 0.33 ± 0.15 , 0.27 ± 0.11 , and 18 ± 2.5 mg/kg, respectively. The researchers attributed the situation to excessive environmental contamination with heavy metals (Al-Rudha et al., 2021). Ribeiro et al. (2021) noted that dairy products from the market in San Luis were a source of heavy metal (Hg, Pb, Se, Cu, Ni) contamination (Ribeiro et al., 2021; Sena et al., 2023). Therefore, we believe that one of the main tasks of cow milk production specialists is the timely and high-quality prediction of the level of contamination of HM feeds and the environmental safety of the produced dairy raw materials according to different types of animal feeding. The regression models we developed with the appropriate correlation analysis will help specialists in production conditions.

The problem of ecosystem pollution is associated with the toxicity of HMs, which have a harmful effect on living organisms. The determining indicator of

the negative impact of chemicals is concentration. It is the concentration of pollutants that we included in our regression model. The highest priority for chemical-toxicological analysis is Pb and Cd, which exhibit high toxicity and migration ability. This has been proven by our studies, including previous research on predicting the transition of HMs from feed rations to organic waste (Portyannik, 2024a) and studies by other scientists. During the experiment, we determined the concentration of copper and zinc in the soil and feed rations, but in this publication we chose the most ecocidal and dangerous Pb and Cd for analysis. In subsequent works, analysis of copper and zinc will be performed. The behavior of ecotoxicants in various natural environments is determined by their specificity and corresponding properties: toxicity, accumulation, mobility, complexation, etc.

Thus, having established a correlation and obtained the corresponding regression equations using a mathematical model, it is possible to predict the corresponding ecological changes and assess the ecological risks in this case of contamination of animal rations with BM in the process of environmentally safe milk production technology. Our research took into account the experience and methodology of both domestic and foreign researchers. The regression equations calculated and tested by us will help to predict the concentration of BM in the rations of different types of feeding of dairy cows and, therefore, to predict the risk of contamination of produced milk with pollutants depending on the amount of toxicants that will enter the animal body with the feed. In the post-war period, there will be a restoration of agricultural production in Ukraine, an increase in the number of cattle, including dairy cows; milk production will increase significantly. Therefore, modeling the body load of dairy cows that will arrive with feed rations in agroecosystems that have been subjected to technogenical or military pollution will be of great importance, especially in the conditions of organic-biological farming and production of environmentally safe livestock and crop products. This study has some limitations, including a small sample size and the lack of independent validation of the model. Future studies may address these limitations.

Conclusions

A positive close relationship and a high correlation between the concentration of cadmium and lead in the soils of agroecosystems and the feed of dairy cows with silage-root crops, silage-hay, silage-haylage, silage-haylage-concentrate type of feeding was established. A very high correlation was found for the cadmium content when feeding animals with silage-haylage-concentrate rations and lead in silage-hay. To predict the concentration of toxic heavy metals in the feed of diets of different types of dairy cows' feeding and to assess the environmental safety of the feed

factor, the corresponding linear regression equations were constructed. The most acceptable models that will give the most accurate prediction result for cadmium in cows on silage–root crops, silage–haylage and silage–haylage–concentrate type of feeding, and for lead in silage–hay and silage–haylage.

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Preliminary *in vivo* Evaluation of Hydroalcoholic *Eucalyptus camaldulensis* Extract as an Alternative Intramammary Therapy for Bovine Mastitis

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Abstract. Bovine mastitis is an inflammatory condition of the mammary gland that induces physical, chemical, and bacteriological alterations in milk, as well as structural changes in mammary tissue. These impacts highlight the need for practical, viable, and cost-effective therapeutic alternatives. The objective of this exploratory, preliminary study was to assess the potential efficacy of a hydroalcoholic extract of *Eucalyptus camaldulensis* as an alternative antimicrobial treatment for bovine mastitis. The sample consisted of 40 quarters with mastitis corresponding to 10 lactating cows, divided into two groups of 20 quarters in the control group (5 cows) and 20 (5 cows) in the group treated with the application of three intramammary doses of the *E. camaldulensis* extract. CMT scores showed no statistically significant differences between the control and treatment groups, either before or 48 hours after the third administration (OR = 0.63; 95% CI: 0.11–3.70; $P = 0.610$). Evaluation of the antimicrobial activity of *E. camaldulensis* extract demonstrated a significant reduction ($P < 0.001$) in bacterial load 24 hours after the third intramammary application. This effect was not observed in the control group ($P \geq 0.05$), as determined by the marginal means test of the mixed-effects model. Based on the findings of this pilot study, the hydroalcoholic extract of *E. camaldulensis* demonstrated a reduction in bacterial load, suggesting possible *in vivo* antimicrobial activity. However, because somatic cell count (SCC) was not measured and inflammatory response was assessed only through the California Mastitis Test (CMT), the study cannot determine whether the treatment influenced mammary inflammation or clinical resolution of mastitis.

Introduction

Bovine mastitis is an inflammation of the mammary gland that can lead to physical, chemical, and bacteriological alterations in milk, as well as structural changes in glandular tissue (Goulart, 2022). From a clinical perspective, clinical mastitis is characterized by visible signs of inflammation of the mammary gland, such as redness and swelling, as well as the presence of clots or other macroscopic changes in the milk, which may be accompanied by more severe systemic signs in acute cases. In contrast, subclinical mastitis does not present overt clinical signs but is associated with an increased somatic cell count and a reduction in milk yield, making it difficult to detect without specific diagnostic tests such as somatic cell counting or the CMT (Morales-Ubaldo, 2023). Intramammary inflammation is associated with an increase in somatic cell count (SCC) in milk, and the magnitude of this increase varies depending on the bacterial species involved. Because SCC is a key marker of inflammatory status and recovery, the absence of post-treatment SCC measurements in the present study precludes an objective assessment of inflammatory resolution (Ramuada, 2024).

Epidemiologically, the subclinical form is far more

prevalent than the clinical form, making it a major challenge for diagnosis and treatment. The absence of visible clinical signs necessitates the use of diagnostic methods for early pathogen identification and the selection of targeted therapies, which in turn may help address the increasing emergence of antimicrobial-resistant bacterial strains. In addition, continuous monitoring and the exploration of alternative therapeutic approaches are required to improve udder health and treatment efficiency (Tommasoni, 2023).

Bovine mastitis is one of the most common diseases in dairy herds and represents one of the greatest economic burdens for the dairy industry. It may be present in clinical or subclinical forms. Factors such as general animal management practices and improper milking procedures, whether performed manually or mechanically, facilitate the harmful activity of common pathogenic microorganisms, including *Streptococcus agalactiae*, *Staphylococcus aureus*, *Streptococcus uberis*, *Klebsiella* spp., *Escherichia coli*, and *Enterococcus* spp. (Morales-Ubaldo, 2023).

The inappropriate use of antibiotics in veterinary medicine poses risks to both human and animal health due to the development of antimicrobial resistance, as observed in *Staphylococcus aureus* and its resistance to penicillin. One of the major concerns of the World Health Organization (WHO) and the World

Organization for Animal Health (WOAH) is the growing bacterial resistance to antibiotics, as well as the increasing costs this imposes on livestock production and the residual effects on consumers. This issue has stimulated research aimed at identifying viable, practical, and cost-effective therapeutic alternatives (Ul-Hamid, 2023). Among these alternatives, natural compounds have gained attention for their ability to stimulate innate defense mechanisms and reduce mammary tissue inflammation, thereby promoting healing and enhancing the resilience of the affected tissue (Askari, 2025).

Eucalyptus was introduced to the Americas by Europeans in the late nineteenth century and has since been used for its medicinal properties to treat numerous diseases, including those of bacterial origin. This plant contains polyphenolic compounds, flavanones, and hydrolysable tannins, which confer strong antioxidants and antimicrobial activity. Recent studies have demonstrated its antimicrobial efficacy against *S. aureus*, *Klebsiella* spp. and *E. coli* (Uriol Plasencia, 2019), and depending on the concentration of *E. camaldulensis* extract, bacteriostatic or bactericidal effects can be obtained against different strains of *S. aureus* (Salari, 2006).

Currently, the use of medicinal plants as alternative treatments for mastitis has not been widely implemented, despite the limited reports of bacterial resistance to plant-derived products. In Nicaragua, there is previous evidence demonstrating the *in vitro* antimicrobial capacity of the hydroalcoholic extract of *Eucalyptus globulus* (Flores-Somarriba, 2021). However, its potential antimicrobial effect has not yet been preliminarily explored *in vivo* in cows with mastitis, and its cytotoxicity remains unknown. The objective of this exploratory, preliminary study was to assess the potential efficacy of a hydroalcoholic extract of *E. camaldulensis* as an alternative antimicrobial treatment for bovine mastitis. Given the absence of somatic cell count measurements and the short follow-up period, the study was not designed to fully evaluate the inflammatory component of mastitis and should therefore be interpreted as generating preliminary hypotheses rather than confirmatory evidence.

Material and Methods

Preparation of the *Eucalyptus camaldulensis* Extract

Two kilograms of *E. camaldulensis* leaves were collected and washed with 5 L of distilled water to remove extraneous substances. The leaves were then air-dried on metal trays at room temperature and under shade for 7 days. Subsequently, 400 g of the dried leaves were weighed using a digital balance and ground with a conventional blender to obtain a fine powder.

A volume of 1800 mL of 70% (v/v) ethyl alcohol was measured and poured into an Erlenmeyer flask

containing the powdered plant material. The mixture was covered with aluminum foil to prevent evaporation and allowed to macerate for 24 h. After this period, the extract was filtered through Whatman No. 1 filter paper into another Erlenmeyer flask. Simultaneously, 100 mL of 70% (v/v) ethyl alcohol was placed in a separate Erlenmeyer flask as a control. Both flasks were left uncovered at room temperature for 7 days. As a final step to remove residual particles, the extract was transferred to a particle separation funnel and left for 48 h until a clear, impurity free liquid was obtained (Flores-Somarriba, 2021). To ensure bacterial sterility prior to *in vivo* application, the extract was inoculated onto tryptic soy agar and 5% blood agar and incubated aerobically for 48 h at 35°C.

Evaluation of the Cytotoxicity of the Extract

A 1% suspension of human red blood cells was prepared. From this suspension, 100 µL was mixed with 100 µL of the hydroalcoholic extract of *E. camaldulensis* at concentrations of 200, 100, 50, 25, 12.5, and 6.75 mg/mL. Simultaneously, a control was prepared by mixing 100 µL of the erythrocyte suspension with 100 µL of saline solution (0.85% NaCl). All mixtures were incubated at 37°C for 30 min and subsequently examined under a microscope to assess the presence of hemolysis in comparison with the control. A cytopathic effect was considered present at concentrations showing hemolysis (Meza et al., 2016).

Determination of Bovine Mastitis

Somatic cell count (SCC) was not measured in this study due to logistical constraints; therefore, inflammatory status was evaluated only using the CMT, which served as a qualitative field screening tool. Subclinical mastitis screening was performed using the CMT. Prior to sampling, the mammary quarters were cleaned with a disinfectant, dried with disposable paper towels, and disinfected with 70% alcohol. The first streams of milk were discarded, and one to two streams per quarter were collected onto the CMT paddle. Subsequently, an equal volume of CMT reagent was added, mixed, and the presence of gel formation was evaluated as an indicator of subclinical mastitis. The paddle was rinsed between animals to prevent cross-contamination (Ranasinghe, 2021).

To confirm mastitis and identify the bacterial agent, the samples were cultured aerobically on 5% blood agar. Identification was carried out based on colony characteristics, Gram staining, catalase test, growth on mannitol salt agar, and coagulase tests on slide and in tube (Mahamed, 2023).

Experimental Design

A controlled and randomized experimental study was conducted. Forty quarters from 10 cows were included; the cows were selected based on

a previous study to ensure that all four quarters presented mastitis according to the CMT and had intramammary infection confirmed by *Staphylococcus aureus*. In the experimental group, each of the four mammary quarters (20 quarters in total) received an intramammary inoculation of 5 mL of a hydroalcoholic extract of *E. camaldulensis* at a concentration of 50 mg/mL. Treatments were administered three times at 7-day intervals (days 0, 7, and 14).

In the control group, each mammary quarter received 5 mL of sterile saline according to the same administration schedule as the treatment group. To evaluate changes in bacterial load, *Staphylococcus* counts were quantified using Petrifilm® Staph Express Count Plates both before the first treatment and 24 h after the third treatment (Nagasawa, 2020). Additionally, the CMT was performed prior to treatment initiation and again 48 h after the third treatment.

Because this study was designed as a preliminary pilot investigation, no formal sample size calculation was performed. Consequently, the number of animals included was limited and the study should be considered statistically underpowered for confirmatory inference.

Statistical Analysis

To evaluate treatment efficacy, CMT scores, treated as an ordinal repeated outcome variable, were analyzed using a cumulative logit ordinal mixed-effects regression model. The model included fixed effects for group (treatment vs. control), time (baseline vs. post-treatment), and their interaction. To account for the clustered and repeated-measures structure of the data, cow was included as a random effect.

Odds ratios (ORs) and their 95% confidence intervals (95% CIs) were estimated from the model. Predicted probabilities for each CMT score category were calculated based on the fitted model. Statistical significance was set at $P < 0.05$. In contrast, bacterial load was analyzed using a linear mixed-effects model, including the experimental group, sampling time, and their interaction as fixed effects, and cow as a random effect, with mammary quarters nested within each cow. The model was fitted by restricted maximum likelihood (REML). Adjusted marginal means were estimated, and post hoc comparisons were performed when appropriate. Analyses were conducted in excel and R (R Foundation for Statistical Computing, Vienna, Austria).

Ethics Statement

The research protocol was approved by the Department of Veterinary Medicine and Animal Science. All procedures were performed by highly qualified veterinarians affiliated with the Universidad Internacional Antonio de Valdivieso (UNIAV). The study was conducted in accordance with Law No. 747, which regulates the protection and welfare of

domestic and domesticated wild animals.

Results

Hemolytic Activity of the Extract

Hemolytic activity was detected at extract concentrations of 200 and 100 mg/mL. No hemolytic activity was observed at lower concentrations.

CMT Scores: Ordinal Regression Analysis

The cumulative logit ordinal regression model showed a non-significant tendency toward higher CMT scores in the treatment group compared with the control group (OR = 3.46; 95% CI: 0.94–12.70; $P = 0.061$). The main effect of sampling time (baseline vs. follow-up) was not statistically significant (OR = 1.59; 95% CI: 0.28–8.82; $P = 0.599$). Likewise, the Group \times Time interaction was not statistically significant (OR = 0.63; 95% CI: 0.11–3.70; $P = 0.610$).

Predicted Probabilities of CMT Scores

In the control group at baseline, the highest predicted probability corresponded to CMT score 2 (0.59; 95% CI: 0.46–0.72), followed by CMT score 3 (0.25; 95% CI: 0.08–0.42) and CMT score 1 (0.16; 95% CI: 0.03–0.29). After follow-up, the predicted probability of CMT score 2 remained the highest (0.59; 95% CI: 0.47–0.72), followed by CMT score 1 (0.23; 95% CI: 0.06–0.41) and CMT score 3 (0.18; 95% CI: 0.03–0.32).

In the treatment group at baseline, the predicted probability was highest for CMT score 2 (0.50; 95% CI: 0.33–0.67), followed by CMT score 3 (0.42; 95% CI: 0.21–0.63) and CMT score 1 (0.08; 95% CI: 0.001–0.16). After treatment, predicted probabilities were 0.49 (95% CI: 0.31–0.66) for CMT score 2, 0.43 (95% CI: 0.21–0.66) for CMT score 3, and 0.08 (95% CI: 0.001–0.15) for CMT score 1 (Fig. 1).

Bacterial Load Analysis

The linear mixed-effects model revealed a significant effect of treatment group on bacterial load ($\beta = -0.590$; SE = 0.164; $z = -3.61$; $P < 0.001$). The main effect of sampling time was not statistically significant ($\beta = 0.064$; SE = 0.114; $z = 0.56$; $P = 0.576$). However, a significant Group \times Time interaction effect was observed ($\beta = 0.440$; SE = 0.161; $z = 2.74$; $P = 0.006$), (Table 1).

Estimated marginal means indicated that, prior to treatment, bacterial load was 4.12 log₁₀ in the treatment group and 4.27 log₁₀ in the control group, with no statistically significant difference between the groups (difference = -0.15 log₁₀; 95% CI: -0.42 to 0.12; $P = 0.28$). After treatment, bacterial load was 3.62 log₁₀ in the treatment group and 4.21 log₁₀ in the control group. At this time point, the difference between the groups was -0.59 log₁₀ (95% CI: -0.91 to -0.27 ; $P < 0.001$), (Fig. 2).

For the mixed-effects model, variance attributable

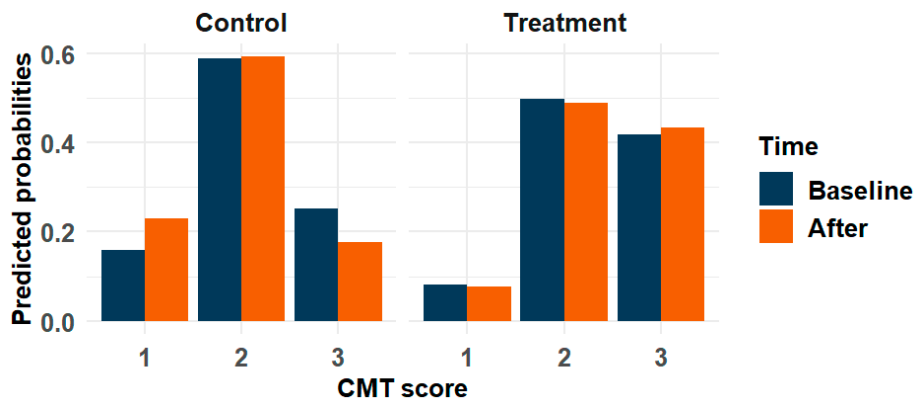


Fig. 1. Predicted probabilities of CMT scores in cows with subclinical mastitis at baseline and 48 hours after treatment with saline solution (control) or *Eucalyptus camaldulensis* (treatment)

Table 1. Estimates of fixed effects on bacterial load

Effects	Estimation (β)	Standard error	z value	P-value
Intercept (Control – After)	4.206	0.116	36.33	<0.001
Group (Treatment vs. Control)	-0.590	0.164	-3.61	<0.001
Time (Before vs. After)	0.064	0.114	0.56	0.576
Group x Time	0.440	0.161	2.74	0.006

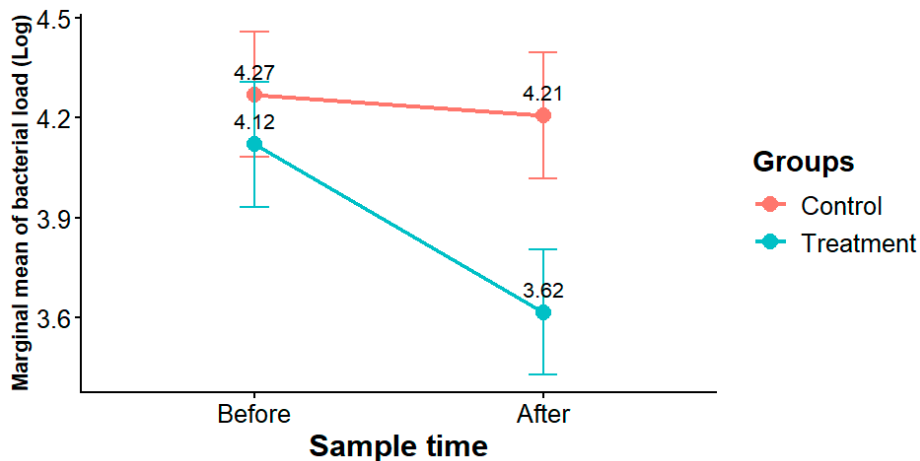


Fig. 2. Estimated marginal means of bacterial load before and after treatment (24 h after the third application) in the control and treatment groups. Points represent the estimated marginal means, and error bars indicate 95% confidence intervals.

to differences between cows was estimated at 0.035, whereas residual variance was 0.129.

Discussion

The present pilot study explored the potential antimicrobial effect of a hydroalcoholic extract of *E. camaldulensis* in cows with mastitis. The main finding was a reduction in bacterial growth after treatment, suggesting that the extract may exert antimicrobial activity under field conditions.

The cytotoxicity findings of the present study indicate that the hydroalcoholic extract of *E. camaldulensis* does not induce a cytopathic effect at concentrations below 50 mg/mL. Notably, this

study represents one of the few investigations to assess *in vitro* cytotoxicity of a plant-derived product intended for the treatment of subclinical mastitis. The evaluation of *in vitro* cytotoxicity constitutes a critical prerequisite prior to intramammary application, as it ensures the absence of deleterious effects on mammary gland cells. This approach is consistent with the study by Kaseke (2023), who conducted preliminary toxicity assessments before performing *in vivo* trials, thereby adhering to fundamental principles of animal welfare and ethical research.

The lack of observable changes in CMT scores may be partially explained by the temporal dynamics of the inflammatory response within the mammary gland.

In mastitis, inflammatory indicators often require longer periods to return to baseline levels following treatment, particularly when tissue repair and immune regulation are still ongoing. In the present study, the assessment of inflammation was limited to CMT and did not include somatic cell count (SCC), which is considered a more sensitive indicator of leukocyte infiltration associated with mammary inflammation. Consequently, inflammatory markers regulated by immune mediators may remain elevated during the early post-treatment period, as the mammary immune response may not have fully resolved within 48 h after treatment (Peckler, 2025).

This interpretation is consistent with previous studies reporting reductions in inflammatory indicators only after longer observation periods. For example, Zelaya Mendoza (2017) reported a decrease in somatic cell counts in cows with mastitis following intramammary administration of a 20% *Hamelia patens* extract after a longer treatment period. Although anti-inflammatory properties have been described for essential oils of *E. camaldulensis* in experimental models (Mondal, 2021), the present study did not directly evaluate the inflammatory response. Therefore, no conclusions can be drawn regarding potential anti-inflammatory effects of the hydroalcoholic extract evaluated here.

The reduction in bacterial presence observed after intramammary administration of the *E. camaldulensis* extract suggests that the formulation may exert antimicrobial activity within the mammary gland environment. The antimicrobial activity observed is consistent with previous studies reporting inhibitory effects of *E. camaldulensis* extracts against bacterial pathogens associated with bovine mastitis, particularly *Staphylococcus* species. Experimental studies have demonstrated that extracts and essential oils of this species can inhibit the growth of *S. aureus* and other pathogenic bacteria through the action of bioactive phytochemicals such as phenolic compounds and terpenoids (Al-Hadid, 2022; Aouadhi, 2024). Furthermore, previous *in vitro* research conducted in Nicaragua reported promising antimicrobial activity of *Eucalyptus* against *S. aureus* isolates from bovine mastitis (Flores-Somarriba, 2021), supporting the findings of the present study.

The antimicrobial properties of *Eucalyptus* are attributed to its complex chemical composition, which includes cineole, terpene hydrocarbons, aldehydes, ketones, terpenes, terpenoids (e.g., 1,8-cineole, carvacrol), and aromatic compounds such as cinnamaldehyde and eugenol. The bactericidal activity of these compounds has been associated mainly with phenols and monoterpenes, which can interact directly with the bacterial cytoplasm or, due to their hydrophobic nature, integrate into the

lipid components of the bacterial cell membrane. This interaction results in increased membrane permeability, leading to ion leakage and subsequent cell death (Montero-Recalde, 2019).

Several methodological limitations should be considered when interpreting these findings. First, the study included a relatively small sample size (five cows per group), and because it was designed as a pilot trial, it is statistically underpowered to detect modest treatment effects or to support confirmatory conclusions regarding clinical efficacy. Second, the inflammatory response of the mammary gland was not directly evaluated because somatic cell count (SCC), the standard quantitative indicator of mammary inflammation, was not measured (Huang, 2022). Instead, inflammation was assessed using the CMT, a practical screening tool that provides only a semi-quantitative estimate of milk cellularity and cannot replace direct SCC measurements. Finally, the follow-up period was limited to 48 hours after the final intramammary administration, which is likely insufficient to evaluate the resolution of mammary inflammation or the longer-term recovery of the mammary gland. Moreover, because bacterial load was assessed only 24 hours after the third treatment, the persistence of the antimicrobial effect could not be determined. Consequently, the present findings should be interpreted cautiously and considered hypothesis-generating rather than definitive evidence of treatment effectiveness.

Conclusion

Within the scope of this pilot study, the hydroalcoholic extract of *E. camaldulensis* demonstrated an acceptable *in vitro* safety profile at concentrations below 50 mg/mL, suggesting potential suitability for intramammary application. Although no significant changes in CMT scores were observed within the 48-hour post-treatment period, a reduction in bacterial load was detected in cows with mastitis, indicating possible *in vivo* antimicrobial activity. However, because somatic cell count (SCC) was not measured and inflammatory response was assessed only through CMT, the present study cannot determine whether the treatment promoted resolution of mammary inflammation or clinical recovery from mastitis. Because of the small number of animals included, the study is underpowered for confirmatory inference, and the results should therefore be interpreted as hypothesis-generating observations that require validation in larger, adequately powered trials. These findings provide an initial basis for further investigation of *E. camaldulensis* as a potential natural antimicrobial candidate for the management of bovine mastitis.

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Morphological Assessment of Arab-Barb Horses in Algeria Based on Linear Measurements, Body Circumferences, and Morphometric Indices

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Abstract. The aim of this study was to define selected morphometric characteristics, namely linear measurements, body circumferences, and biometric indices, of Arab-Barb horses across different localities in Algeria. A total of twenty linear measurements were selected for morphometric analysis, and ten indices or body ratios were calculated from 49 Algerian Arab-Barb horses. The descriptive results indicated a relatively homogeneous population in northern Algeria based on the measured morphometric variables. Sex-related differences were observed mainly for neck length, neck circumference, and knee circumference ($P < 0.05$). Horses in the > 480 kg estimated body weight (BW) group showed greater total length, humero-iliac length, chest circumference, withers height, rump height, cannon circumference, fetlock circumference, and several mass-related indices. Significant differences were observed in 14 linear measurements of Algerian horses between two studied regions ($P < 0.05$). Most correlations among morphological variables exceeded 0.6, suggesting strong interrelationships and morphological consistency. The dactyl thorax index was significantly lower in females than in males ($P < 0.05$), whereas thoracic development was higher in females in the unadjusted comparison. Overall, the findings indicate relative morphometric homogeneity in Arab-Barb horses from northern Algeria, while also highlighting variation associated with sex, estimated body weight, and geographic origin. In the future, molecular analysis of Algerian horses will be essential for the preservation and management of this invaluable genetic resource, which constitutes an integral part of the nation's cultural and economic heritage.

Introduction

In Algeria, equestrian sports have developed considerably in recent years in terms of the number of horses and horse owners. According to the 2019 national inventory, the horse population was estimated at 312 000 heads nationwide (Ministry of Agriculture and Rural Development, 2019). Algeria thus ranks as the second-largest country in North Africa, after Morocco, in terms of horse population. Horses are distributed across many northern regions of Algeria, primarily in highland and mountainous areas. Two local horse breeds predominate in Algeria: the Barb and the Arab-Barb, alongside several imported breeds, including the Arabian Thoroughbred, English Thoroughbred, French Trotter, and Selle Français.

The Barb horse has played a significant role in North Africa's history, particularly in resistance wars, as well as in the economy through transport and agriculture. It is also used in the Fantasia – a traditional equestrian performance showcased during

cultural festivals. This breed has been exported for centuries because of its hardiness and exceptional endurance (Van der Merwe and Martin, 2002). The Arab-Barb horse originated at the Tiaret stud farm in western Algeria in 1877, when the local Barb breed was crossed with Arabian horses to meet the military cavalry's requirements and to improve certain characteristics of the Barb horse (Thompson, 1995). According to the studbook of the World Barb Horse Organization, horses resulting from these crossings are referred to as Arab-Barb horses (Rahal et al., 2009). As this breed spread throughout Algeria (Kabylie, Saoura, Sersou, Titteri, Dahra, El Hodna, and the Aurès region), variations in Barb morphology emerged. Therefore, it is important to continuously evaluate the morphometric characteristics of Algerian horse breeds in order to establish more accurate criteria for their definition and characterization.

The knowledge of morphometric measurements in horses is crucial for taxonomic classification, breed development, and the maintenance of genetic diversity. In Algeria, relatively few studies have been conducted on the morphometric characteristics of

Arab-Barb horses (Behamadi et al., 2017; Benia et al., 2022; Mebarki et al., 2018; Rahal et al., 2009), despite the numerous risks associated with genetic drift and breed modification. Therefore, the main objective of the present study was to improve knowledge of Algerian horse breeds. The investigation aimed to define certain morphological variables, including linear measurements, body circumferences, and biometric indices, in Arab-Barb horses from different regions of Algeria. In addition, Pearson correlation coefficients among the various linear measurements were evaluated.

Material and Methods

For animal experimentation, ethical review and approval were waived for this study because the morphometric measurements were performed with the consent of the horse owners. The data collection procedure was non-invasive and did not cause additional suffering to the animals.

Study region

The study was conducted from March to July 2022 in the Bejaia (36°45'N, 5°3'E) and Oran (35°41'N, 0°38'W) regions of Algeria. Both regions belong to the Mediterranean climatic zone, characterized by mild to warm and rainy winters, frequent precipitation mainly during autumn and winter, and

hot and dry in summers.

Animals and measurements

A total of 49 Algerian Arab-Barb horses aged 4 years or older were included. Linear measurements were performed in each stable on a level, and non-slip horizontal surface, using a specially graduated measuring tape. A total of twenty linear measurements were selected for morphometric characterization, as described below:

eye interval (EI): distance between the inner canthi of the eyes; head length (HL): distance between the neck and the tip of the nose; neck length (NL): distance between the middle of the anterior edge of the shoulder and the most noticeable part of the edge of the first cervical vertebra; neck circumference (NC): circumference measured at the base of the neckline; shoulder length (SL): distance between the summit and the tip of the shoulder; total length (TL): distance between the greater tubercle (tuberculum majus) of the humerus and the ischial tuberosity (tuber ischiadicum); humero-iliac length (HIL): distance between the greater tubercle of the humerus and the coxal tuber (tuber coxae) of the ilium; chest circumference (ChC): circumference around the chest behind the withers; withers height (WH): vertical distance from the highest point of the withers to the floor; rump height (RH): vertical distance from

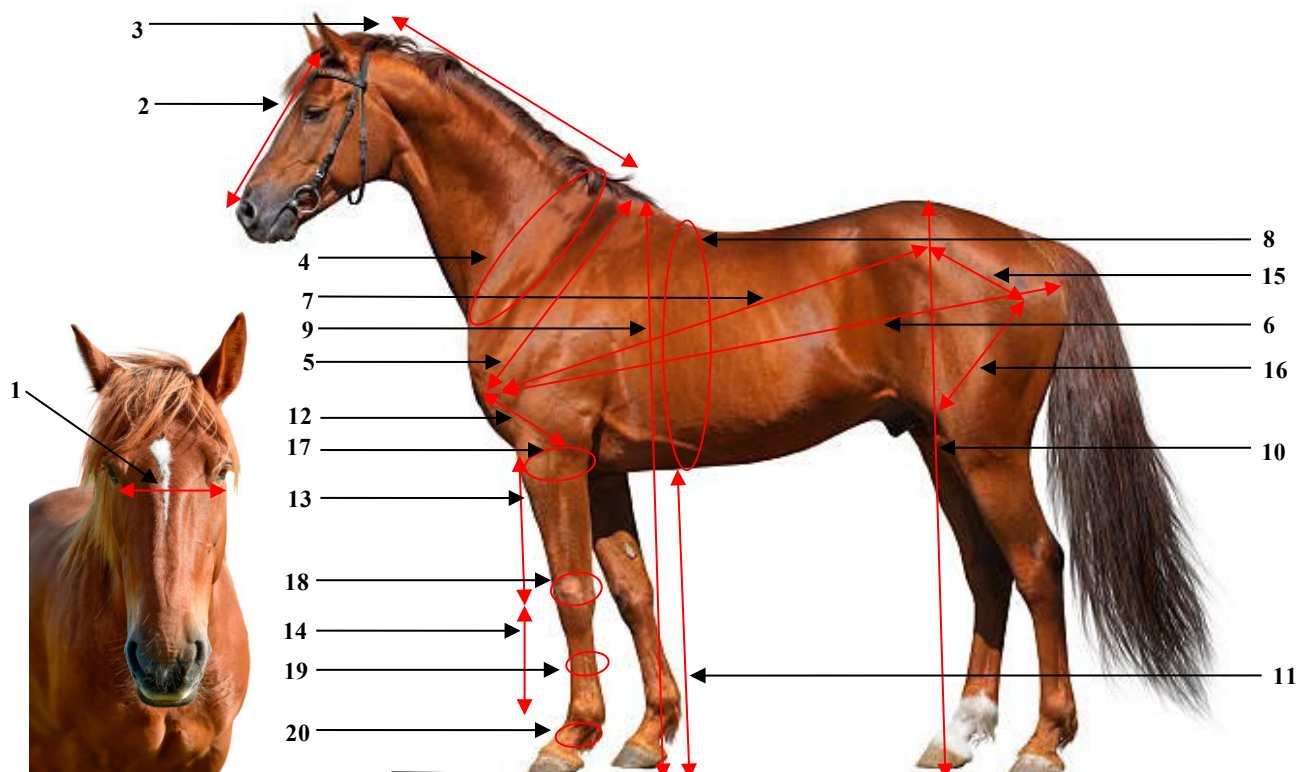


Fig. 1. The different linear measurements performed in horses.

1 – eye interval (EI); 2 – head length (HL); 3 – neck length (NL); 4 – neck circumference (NC); 5 – shoulder length (SL); 6 – total length (TL); 7 – humero-iliac length (HIL); 8 – chest circumference (ChC); 9 – withers height (WH); 10 – rump height (RH); 11 – space under the chest (SC); 12 – arm length (AL); 13 – forearm length (FoL); 14 – cannon length (CL); 15 – ilium length (IL); 16 – femur length (FeL); 17 – forearm circumference (FoC); 18 – knee circumference (KC); 19 – cannon circumference (CC); 20 – fetlock circumference (FeC)

the highest point over the sacral tuberosity to the floor; space under the chest (SC): distance between the sternum and the floor; arm length (AL): distance from the central area of the scapulo-humeral joint to the central area of the humero-radial joint; forearm length (FoL): distance from the central area of the humero-radial joint to the lateral middle third of the carpal joint; cannon length (CL): distance from lateral middle third of the carpal joint to middle third of lateral face of the metacarpo-phalangeal joint of right forelimb; ilium length (IL): distance between the greater trochanter of femur and the coxal tuber of ilium; femur length (FeL): distance between the greater trochanter of femur and the patella; forearm circumference (FoC): midpoint between the distance from the central area of the humero-radial joint and the lateral middle third of the carpal joint; knee circumference (KC): circumference of the central region of the carpus; cannon circumference (CC): circumference of the cannon bone at the middle point of its length; fetlock circumference (FeC): circumference of the middle third of the lateral face of the metacarpo-phalangeal joint.

From the measured morphological variables, body indices and ratios were calculated following established methods (Cabral et al., 2004; Hill et al. 2020; Marcenac and Aubert, 1974; Mariante et al., 2002; McManus et al., 2008; Rezende et al. 2015), as detailed below:

- Body index (BI) = TL/ChC; if BI > 0.90, they are longiline, suited for speedy animals; between 0.86 and 0.88, mediline with midway ratios has intermediate abilities and <0.85, breviline, more apt for force or strength.
- Body ratio (BR) = WH/TL; if the WH is less than the TL, the animal is considered low in front and vice versa, which is considered as defects. This inequality in heights may hinder both gait and resistance.
- Compact index 1 (CI1) = BW/WH; it indicates how compact the horse is. Animals used as saddle horses have CI1 < 2.20, animals suited for light traction have $2.60 \leq CI1 \leq 3.15$, and horses used for heavy pulling/traction have CI1 > 3.15.
- Dactyl thorax index (DTI) = CC/ChC; DTI > 0.105 for light small horses, up to 0.108 in intermediary horses, up to 0.110 in light traction animals, and up to 0.115 in heavy traction and riding horses. This index also indicates thoracic development.
- Relative body index (RBI) = TL/WH; it indicates useful indices in assessing functionality in horses. If > 110 in RBI, animals are overweight with poor gait; meanwhile, animals with an RBI lower than < 90 show poor gait and emaciated muscular appearances.
- Tare index 1 (trot or gallop) (TI1) = $ChC^2 \times 56/WH$; this index indicates weight in kilograms that the animal can withstand without excessive stress on the back, which can be carried working at a trot or gallop.

- Tare index 2 (walk) (TI2) = $ChC^2 \times 95/WH$; this index indicates the weight in kilograms that the animal can carry at a walking pace without excessive stress on its back while working at a walking speed.
- Thoracic development (TD) = ChC/WH; the values > 1.2 indicate animals with good TD or massiveness. Poor TD is associated with a lack of good musculature and deficiencies in the cardiovascular system. A horse with an excessively large thorax is also not desirable as the centre of gravity moves to the front of the horse.
- Relative thickness of the cannon bone index (RTCI) = CC/WH; higher values indicate harmony between the body conformation and bone development, and vice versa.
- Front-back height in (FBH) = WH/RH; FBH ≤ 1: straight back (no overload), or FBH > 1: the anterior region is higher than the posterior (overload).

The estimated body weight (BW) for each animal was calculated using the equation proposed by Eley and French (1993):

$$BW = (WH^{0.24}) \times (ChC^{2.576}) \times 0.000252$$

As this equation was originally developed for donkeys and has not yet been specifically validated in Arab-Barb horses, the resulting body weight (BW) estimates were considered as operational morphometric indicators rather than precise measures of actual live weight. Consequently, all BW-related analyses were interpreted in terms of comparisons among estimated body mass categories and were used primarily for relative group allocation, rather than as assessments based on exact body weight values.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics, Version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics for all morphometric variables are presented as mean ± standard deviation (SD), minimum and maximum values, median, and coefficient of variation (%). Two-level comparisons of linear measurements and morphometric indices according to sex, estimated body weight group (≤ 480 kg vs > 480 kg), and region (Bejaia vs Oran) were performed using independent-samples t-tests, with Welch's correction when variances were unequal. Each morphometric index or ratio was analyzed separately as the dependent variable in a univariate general linear model (GLM). The model included the morphometric index as the dependent variable. Sex (male vs female), body weight Group (≤ 480 kg vs > 480 kg), and region (Bejaia vs Oran) were entered as fixed factors. The assumptions of the model homogeneity of variance and normality of residuals were checked. Statistical significance was set at P < 0.05, and GLM P values were obtained from Type III Sum of Squares F-tests. Pearson correlation coefficients (r) were calculated to evaluate the linear

relationships between linear measurements, estimated body weight, and morphometric indices. Model selection was based on predefined biological and geographic sources of variation. Interaction terms were not retained to preserve degrees of freedom given the modest sample size. Model fit, and effect magnitude were summarized using R^2 , and 95% confidence intervals were calculated for key descriptive estimates.

Results and Discussion

In general, the use of body morphometric descriptions as a means of comparing different animal breeds is a valuable approach (Ayad et al., 2019; Lomillos et al., 2020), as it provides important information on variation in form. This linear morphological assessment methodology is essential in animal biology and can be used in genetic analysis. It has been observed that animal genetic resources demonstrate significant heterogeneity with respect to breed in the natural environment, particularly with respect to body morphology and production potential. The Algerian horse population is predominantly composed of Barb and Arab-Barb horses, accounting

for 90% of the total, with the remaining 10% comprising a combination of other breeds (FAO, 2021). A clear distinction in the morphometric difference among horse breeds has been demonstrated (Brooks et al., 2010). The overall objective of this study was to highlight a series of morphometric characteristics in horses. These included linear measurements, body circumferences, and body biometric indices of Arab-Barb horses from two Algerian localities.

The results in Table 1 provide a comprehensive overview of the descriptive statistics of morphological variables, including mean, standard deviation, minimum and maximum values, median, and coefficient of variation, from linear measurements taken from Algerian Arab-barb horses. The findings of this study demonstrate that the descriptive analysis of horse linear measurements in northern Algeria exhibits relatively homogeneous patterns, as evidenced by coefficient of variation (CV) that does not exceed 0.23. Measurements of withers height, hind limb length, heart girth, body length, and rump length have been shown to provide valid estimates of total body size (Birteeb et al., 2012). This is because they

Table 1. Descriptive analysis of horse linear measurements in northern Algeria

Linear measures	Mean \pm SD	Min-Max	Median	CV (%)	95% CI
BW (kg)	485.0 \pm 66.9	336.1–631.6	493.9	13.8	465.7–504.1
EI (cm)	14.5 \pm 3.5	6–17	16	24.1	13.5–15.5
HL (cm)	58.9 \pm 3.4	50–66	60	5.8	57.9–59.9
NL (cm)	62.7 \pm 5.4	53–80	62	8.6	61.1–64.3
NC (cm)	108.7 \pm 12.0	93–150	107	11.1	105.3–112.1
SL (cm)	60.7 \pm 3.5	55–69	60	5.7	59.7–61.7
TL (cm)	145.8 \pm 7.9	134–169	145	5.5	143.5–148.1
HIL (cm)	109.2 \pm 6.7	95–124	110	6.1	107.3–111.1
ChC (cm)	171.9 \pm 9.1	151–190	173	5.3	169.3–174.5
WH (cm)	151.5 \pm 6.8	136–162	153	4.5	149.6–153.4
RH (cm)	150.4 \pm 5.7	138–160	152	3.8	148.8–152.0
SC (cm)	83.1 \pm 5.7	70–99	84	6.9	81.5–84.7
AL (cm)	30.0 \pm 5.2	21–46	28	17.5	28.5–31.5
FoL (cm)	40.3 \pm 3.2	31–47	40	8.0	39.4–41.2
CL (cm)	26.4 \pm 2.1	22–31	26	8.1	25.8–27.0
IL (cm)	38.4 \pm 8.6	26–55	35	22.3	35.9–40.9
FeL (cm)	38.9 \pm 6.6	26–53	38	17.1	37.0–40.8
FoC (cm)	45.5 \pm 4.0	40–54	44	8.7	44.4–46.6
KC (cm)	29.6 \pm 2.2	25–34	30	7.3	29.0–30.2
CC (cm)	19.3 \pm 1.2	17–22	19	6.0	19.0–19.6
FeC (cm)	24.1 \pm 4.0	15–30	25.5	16.7	23.0–25.2

Estimated body weight (BW); eye interval (EI); head length (HL); neck length (NL); neck circumference (NC); shoulder length (SL); total length (TL); humero-iliac length (HIL); chest circumference (ChC); withers height (WH); rump height (RH); space under the chest (SC); arm length (AL); forearm length (FoL); cannon length (CL); ilium length (IL); femur length (FeL); forearm circumference (FoC); knee circumference (KC); cannon circumference (CC); fetlock circumference (FeC); coefficient of variation (CV); standard deviation (SD), CI, 95% confidence interval around the whole-sample mean.

include key linear measurements that are indicative of body conformation and overall mass. To enhance the reporting of estimate precision, 95% confidence intervals (95% CI) were calculated for the principal morphometric and body weight variables across the entire study population. The corresponding intervals were 465.7–504.1 kg for BW, 169.3–174.5 cm for ChC, 149.6–153.4 cm for WH, 143.5–148.1 cm for TL, and 19.0–19.6 cm for CC.

The study results demonstrated that the size of the horses, based on height at the withers, overall length, and cannon bone circumference, was consistent with the results obtained in previous studies of different localities in Algeria (Rahal et al., 2009; Mebarki et al., 2018; Madani et al., 2024). The significant contribution of withers height and rump dimensions underscores the importance of the general skeletal framework in distinguishing breeds. The configuration of the horse's physique is indicative of its robustness. It is noteworthy that Arab-Barb horses are distinguished by their robustness and high adaptability, which enables their use across diverse activities such as ceremonial parades, field work, and

training applications. Additionally, they are used as a component of fantasias that constitute a conventional exposition of equestrian expertise in the Maghreb, which is performed during cultural festivals. As Denoix (2002) notes, the neck of the racehorse must be of sufficient length to ensure effective balance function, particularly during galloping, without overloading the front hand.

The data regarding the average weight and size of the animals' withers indicate a small body format, as noted by Benia et al. (2022). Moreover, it has been demonstrated that during gallop races, larger body surfaces require the use of enhanced thermoregulatory mechanisms (Metayer et al., 2003). On the other hand, Giontella et al. (2024) have reported higher values for wither height and cannon bone circumference (164.31 ± 0.20 and 20.78 ± 0.06 , respectively). In a recent study performed on Algerian fantasia horses, the values of withers height and body length were lower than those reported by Chaabi et al. (2025) (155.2 ± 2.76 and 154.3 ± 4.38 cm, respectively). The measurement of cannon circumference is indicative of the thickness of the bones and limb strength,

Table 2 Values of morphometric linear measurements of Algerian horses according to sex

Linear measures	Female (n = 27) (Mean \pm SD)	Male (n = 22) (Mean \pm SD)	P Value
BW (kg)	497.3 \pm 71.7	470.0 \pm 58.6	0.158
EI (cm)	14.6 \pm 3.2	14.2 \pm 3.9	0.679
HL (cm)	58.2 \pm 3.4	59.7 \pm 3.2	0.125
NL (cm)	60.6 \pm 2.8	65.2 \pm 6.6	0.005
NC (cm)	104.4 \pm 6.9	113.9 \pm 14.8	0.01
SL (cm)	60.6 \pm 3.1	60.8 \pm 3.9	0.823
TL (cm)	146.3 \pm 8.6	145.2 \pm 7.2	0.63
HIL (cm)	108.8 \pm 7.1	109.6 \pm 6.2	0.673
ChC (cm)	173.6 \pm 9.8	169.9 \pm 8.0	0.155
WH (cm)	151.0 \pm 6.3	152.1 \pm 7.4	0.592
RH (cm)	150.5 \pm 5.0	150.4 \pm 6.5	0.965
SC (cm)	82.5 \pm 5.3	83.8 \pm 6.3	0.437
AL (cm)	29.2 \pm 5.0	30.9 \pm 5.5	0.267
FoL (cm)	40.1 \pm 3.2	40.5 \pm 3.4	0.644
CL (cm)	26.0 \pm 2.0	26.8 \pm 2.2	0.183
IL (cm)	38.3 \pm 8.1	38.5 \pm 9.3	0.909
FeL (cm)	38.7 \pm 6.1	39.1 \pm 7.4	0.841
FoC (cm)	45.0 \pm 3.8	46.2 \pm 4.2	0.305
KC (cm)	28.7 \pm 1.9	30.7 \pm 2.0	< 0.001
CC (cm)	19.0 \pm 1.1	19.7 \pm 1.1	0.054
FeC (cm)	23.8 \pm 4.0	24.5 \pm 4.1	0.537

Estimated body weight (BW); eye interval (EI); head length (HL); neck length (NL); neck circumference (NC); shoulder length (SL); total length (TL); humero-iliac length (HIL); chest circumference (ChC); withers height (WH); rump height (RH); space under the chest (SC); arm length (AL); forearm length (FoL); cannon length (CL); ilium length (IL); femur length (FeL); forearm circumference (FoC); knee circumference (KC); cannon circumference (CC); fetlock circumference (FeC).

traits which are essential for structural soundness and adaptability in rough terrains, particularly in mountainous regions (Yakubu and Ibrahim, 2011). The observed disparities might be related to environmental and management practices, as well as to breed factors or crossbreeding that has occurred over time (Adinata et al., 2023).

Mean values of morphological variables of the Algerian horse population according to sex group are presented in Table 2. Neck length, neck circumference, and knee circumference differed significantly according to sex ($P < 0.05$). These differences indicate limited sexual dimorphism for selected neck and limb measurements. In contrast, estimated BW, head length, chest circumference, most other linear measurements did not differ significantly between females and males in the corrected analysis. The phenotypic differences in body size between the sexes are well documented and are associated with sexual dimorphism, which is driven by hormones that influence growth rates in mammalian taxa (Okpeku et al., 2011). However, in the present data set, sex-related differences should be interpreted cautiously

because the sample size was modest and body weight was estimated rather than directly measured. Values of linear measurements of horses in Algeria according to the estimated BW group are depicted in Table 3. Horses in the > 480 kg estimated BW group showed higher total length, humero-iliac length, chest circumference, withers height, rump height, cannon circumference, and fetlock circumference than horses in the ≤ 480 kg estimated BW group ($P < 0.05$). These results suggest that estimated BW was generally associated with larger thoracic, height, and selected support-structure dimensions. This interpretation must be cautious because BW was estimated partly from WH and ChC, which can strengthen statistical associations between estimated BW group and these measurements.

The estimated BW values obtained using this equation differ from values reported in previous investigations in Algeria (Benhamadi et al., 2017; Guedaoura et al., 2011; Rahal et al., 2009; Kacem et al., 2024). It has been reported that using chest girth and body length as linear measurements provides useful parameters for predicting body weight in cows

Table 3. Values of morphometric linear measurements of Algerian horses according to estimated body weight group

Linear measures	Estimated BW group (≤ 480 kg) (n = 22) (Mean \pm SD)	Estimated BW group (> 480 kg) (n = 27) (Mean \pm SD)	P Value
BW (kg)	426.3 \pm 46.8	532.9 \pm 34.6	< 0.001
EI (cm)	14.0 \pm 3.9	14.8 \pm 3.1	0.412
HL (cm)	58.4 \pm 2.9	59.2 \pm 3.8	0.409
NL (cm)	62.5 \pm 5.3	62.8 \pm 5.6	0.882
NC (cm)	105.2 \pm 8.0	111.4 \pm 14.0	0.071
SL (cm)	60.4 \pm 4.0	60.9 \pm 2.9	0.608
TL (cm)	143.1 \pm 7.0	148.0 \pm 8.1	0.033
HIL (cm)	106.7 \pm 6.3	111.2 \pm 6.3	0.016
ChC (cm)	164.0 \pm 6.7	178.4 \pm 4.5	< 0.001
WH (cm)	148.5 \pm 7.3	154.0 \pm 5.2	0.005
RH (cm)	148.0 \pm 5.9	152.4 \pm 4.7	0.006
SC (cm)	82.5 \pm 7.2	83.5 \pm 4.2	0.594
AL (cm)	30.6 \pm 6.1	29.5 \pm 4.5	0.467
FoL (cm)	39.7 \pm 3.8	40.8 \pm 2.7	0.262
CL (cm)	26.9 \pm 2.0	26.0 \pm 2.2	0.142
IL (cm)	40.8 \pm 8.1	36.4 \pm 8.6	0.078
FeL (cm)	39.7 \pm 6.4	38.2 \pm 6.9	0.449
FoC (cm)	45.3 \pm 3.9	45.7 \pm 4.1	0.766
KC (cm)	29.0 \pm 2.1	30.1 \pm 2.1	0.057
CC (cm)	18.9 \pm 0.8	19.6 \pm 1.3	0.016
FeC (cm)	22.7 \pm 4.4	25.2 \pm 3.4	0.034

Estimated body weight (BW); eye interval (EI); head length (HL); neck length (NL); neck circumference (NC); shoulder length (SL); total length (TL); humero-iliac length (HIL); chest circumference (ChC); withers height (WH); rump height (RH); space under the chest (SC); arm length (AL); forearm length (FoL); cannon length (CL); ilium length (IL); femur length (FeL); forearm circumference (FoC); knee circumference (KC); cannon circumference (CC); fetlock circumference (FeC).

(Paputungan et al., 2013). The differences between the average weight values could be explained by the condition of the horses at the time of measurement, but also by the formulas used to estimate body weight. The body morphometric results of horses by region from the regions of Oran and Bejaia in Algeria are presented in Table 4. Fourteen linear body parameters differed significantly between Oran and Bejaia regions ($P < 0.05$). The observed variation in the size and morphology of horses could be attributed to the geographical environments in which they are found (e.g., coastal plains, mountainous regions, or semi-desert areas). Furthermore, temperature and rainfall have been demonstrated to influence vegetation, which in turn indirectly affects animal development. The species-specific origin of the BW equation should be considered when interpreting these values. Differences in thoracic conformation, body condition, and breed type may influence the accuracy of field-based BW estimates. Moreover, because ChC and WH are included in the equation, analyses involving estimated BW, ChC, or WH are partly mathematically linked and should be interpreted as morphometric

associations rather than independent validation of live weight.

Body indices are important for understanding proportionality, conformation, and breed type and may also indicate environmental adaptations. It has been noted that they play a significant role in the selection of productive and reproductive traits. The most essential aspects for hard work are function, aptitude, disposition, and training (Folla et al., 2020). The results of various indices and ratios derived from linear measurements taken from Algerian Arab-Barb horses are summarized in Table 5. The results of body biometric indices according to sex, estimated body weight, and region are summarized in Tables 6, 7, and 8, respectively. In the unadjusted sex comparison, females showed higher thoracic development (TD), whereas DTI was lower in females than in males ($P < 0.05$). CI1, T11, TI2, RTCI, and the remaining indices did not differ significantly between sexes in the corrected unadjusted comparison.

According to DTI values, the body structure of female horses corresponds to that of light-traction categories, whereas male horses correspond

Table 4. Values of morphometric linear measurements of Algerian horses according to region

Linear measures	Oran region (n = 34) (Mean \pm SD)	Bejaia region (n = 15) (Mean \pm SD)	P Value
BW (kg)	497.2 \pm 46.4	457.2 \pm 95.4	0.141
EI (cm)	16.3 \pm 0.5	10.3 \pm 3.8	< 0.001
HL (cm)	60.1 \pm 2.1	56.1 \pm 4.1	0.002
NL (cm)	64.2 \pm 5.4	59.3 \pm 3.5	0.002
NC (cm)	109.4 \pm 13.0	106.9 \pm 9.6	0.512
SL (cm)	59.4 \pm 2.1	63.5 \pm 4.2	0.002
TL (cm)	144.9 \pm 6.6	147.7 \pm 10.5	0.352
HIL (cm)	108.4 \pm 5.5	110.9 \pm 8.7	0.313
ChC (cm)	173.6 \pm 6.2	168.2 \pm 13.1	0.146
WH (cm)	153.9 \pm 4.1	146.1 \pm 8.4	0.004
RH (cm)	152.4 \pm 3.9	146.0 \pm 6.6	0.002
SC (cm)	84.6 \pm 3.9	79.5 \pm 7.4	0.021
AL (cm)	27.0 \pm 2.4	36.7 \pm 3.5	< 0.001
FoL (cm)	41.5 \pm 2.6	37.5 \pm 2.8	< 0.001
CL (cm)	26.0 \pm 1.9	27.2 \pm 2.4	0.068
IL (cm)	33.4 \pm 4.2	49.6 \pm 4.1	< 0.001
FeL (cm)	35.3 \pm 3.7	47.0 \pm 4.0	< 0.001
FoC (cm)	43.9 \pm 2.9	49.3 \pm 3.6	< 0.001
KC (cm)	30.2 \pm 2.0	28.3 \pm 2.1	0.003
CC (cm)	19.2 \pm 1.1	19.5 \pm 1.2	0.527
FeC (cm)	26.5 \pm 1.7	18.7 \pm 1.9	< 0.001

Estimated body weight (BW); eye interval (EI); head length (HL); neck length (NL); neck circumference (NC); shoulder length (SL); total length (TL); humero-iliac length (HIL); chest circumference (ChC); withers height (WH); rump height (RH); space under the chest (SC); arm length (AL); forearm length (FoL); cannon length (CL); ilium length (IL); femur length (FeL); forearm circumference (FoC); knee circumference (KC); cannon circumference (CC); fetlock circumference (FeC).

Table 5. Morphometric indices and ratios of Algerian horses

Index/Ratio Horses Total (n = 49) (Mean ± SD)			
BI	0.85 ± 0.05	TI1	109.53 ± 10.14
BR	1.04 ± 0.06	TI2	185.82 ± 17.21
CI1	3.20 ± 0.39	TD	1.14 ± 0.06
DTI	0.112 ± 0.007	RTCI	0.128 ± 0.008
RBI	0.96 ± 0.05	FBH	1.01 ± 0.02

Body index (BI); body ratio (BR); compact index 1 (CI1); dactyl thorax index (DTI); relative body index (RBI); tare index 1 (trot or gallop) (TI1); tare index 2 (walk) (TI2); thoracic development (TD); relative thickness of cannon bone index (RTCI); front-back height in (FBH).

Table 6. Morphometric index and ratio of the horses in Algeria according to sex

Index/Ratio	Female (n = 27) (Mean ± SD)	Male (n = 22) (Mean ± SD)	P Value
BI	0.84 ± 0.04	0.86 ± 0.06	0.371
BR	1.03 ± 0.06	1.05 ± 0.06	0.38
CI1	3.29 ± 0.42	3.09 ± 0.33	0.075
DTI	0.110 ± 0.007	0.116 ± 0.005	0.001
RBI	0.97 ± 0.05	0.96 ± 0.06	0.406
TI1	112.03 ± 10.85	106.48 ± 8.46	0.056
TI2	190.04 ± 18.41	180.63 ± 14.36	0.056
TD	1.15 ± 0.06	1.12 ± 0.05	0.046
RTCI	0.126 ± 0.008	0.129 ± 0.009	0.143
FBH	1.00 ± 0.02	1.01 ± 0.02	0.151

Body index (BI); body ratio (BR); compact index 1 (CI1); dactyl thorax index (DTI); relative body index (RBI); tare index 1 (trot or gallop) (TI1); tare index 2 (walk) (TI2); thoracic development (TD); relative thickness of cannon bone index (RTCI); front-back height in (FBH).

Table 7. Morphometric index and ratio of Algerian horses according to estimated body weight

Index/Ratio	Estimated BW (≤ 480 kg) (n = 22) (Mean ± SD)	Estimated BW (> 480 kg) (n = 27) (Mean ± SD)	P Value
BI	0.87 ± 0.04	0.83 ± 0.04	< 0.001
BR	1.04 ± 0.06	1.04 ± 0.06	0.784
CI1	2.87 ± 0.24	3.46 ± 0.27	< 0.001
DTI	0.115 ± 0.006	0.11 ± 0.007	0.006
RBI	0.97 ± 0.05	0.96 ± 0.06	0.794
TI1	101.58 ± 6.06	116.01 ± 7.95	< 0.001
TI2	172.33 ± 10.28	196.81 ± 13.49	< 0.001
TD	1.11 ± 0.04	1.16 ± 0.06	< 0.001
RTCI	0.127 ± 0.007	0.128 ± 0.009	0.917
FBH	1.01 ± 0.02	1.01 ± 0.02	0.135

Body index (BI); body ratio (BR); compact index 1 (CI1); dactyl thorax index (DTI); relative body index (RBI); tare index 1 (trot or gallop) (TI1); tare index 2 (walk) (TI2); thoracic development (TD); relative thickness of cannon bone index (RTCI); front-back height in (FBH).

more closely to heavy-traction and riding horses, respectively. This index may also suggest that thoracic development is more pronounced in male horses than in female horses. Furthermore, CII refers to conformation category, and indicates the type of exercise traditionally associated with the index. The dactyl-thorax index reflects the relationship between body mass of individuals and the limbs that support it. Bone development may be indicated by the dactyl-thorax index and relative thickness of the cannon bone index. The DTI values obtained demonstrated that the horses exhibited sex-related differences in limb-to-thorax proportionality and bone development. These indices are best understood as indirect descriptors of proportionality and support structure. They may help generate hypotheses about functional aptitude, whereas confirmation of athletic performance, endurance capacity, or biomechanical efficiency would require direct measurements of locomotion, physiological, or performance measurements. These results are comparable with those reported in Spain (Solé et al., 2013) and Brazil (de Siqueira and Cordeiro, 2024).

Furthermore, our results revealed that the CII, TI1, TI2, and TD values of horses in the > 480 kg estimated BW group were significantly higher ($P < 0.05$) than those of horses in the ≤ 480 kg estimated BW group. In contrast, BI and DTI showed higher values in the ≤ 480 kg estimated BW group. This pattern appears to reflect the contribution of estimated BW and chest circumference to these indices. Therefore, the observed differences are more appropriately considered morphometric patterns associated with estimated mass than direct indicators of muscularity, endurance capacity, or physical activity exhibited by horses. Likewise, it has been reported that linear measurements and morphometric indices can be used to predict body weight in goats (Putra and Ilham, 2019).

The comparison between horses from the two regions of Algeria showed no significant differences in BI, BR, DTI, and RTCI ($P < 0.05$) was higher in horses from Bejaia region than in Oran region, whereas BR values were lower in Bejaia horses. The horses from the Bejaia area (Kabylie mountains) exhibited ration values closer to the mediline category, whereas Oran horses were closer to the breviline conformation category. These categories should be interpreted as morphological descriptors rather than as direct indicators of strength, traction ability, gait quality, or endurance capacity. This finding is consistent with the observations reported by Madani et al. (2024), who noted significant differences in body indices between horse populations in Algeria. A similar observation has been reported in Algerian Arab-Barb horses, in which body index values vary in different regions (coastal plains, high plateaus, desert, and mountains) (Mebarki et al., 2018). This may be explained by environmental factors, as climatic conditions and

farming systems differ between regions, likely because of their geographical separation.

Separate univariate GLMs were fitted for each morphometric index or ratio to assess the effects of sex, estimated BW group, and region on horse morphometric indices and ratios, as presented in Table 9. Estimated BW group emerged as the primary determinant of compactness and mass-related indices, with horses in the > 480 kg estimated BW group exhibiting significantly higher CII, TI1, TI2, and TD values ($P < 0.001$), independent of sex or region. Region also influenced body proportions, as evidenced by significant differences in BI, BR, DTI, RBI, and RTCI between regions ($P < 0.05$), suggesting environmental or sub-population effects on skeletal conformation. Sex had a comparatively limited impact after controlling for estimated body weight group and region, with DTI remaining significantly associated with sex ($P = 0.003$). Model R^2 values ranged from 0.157 for FBH to 0.593 for CII, indicating variable explanatory capacity depending on the index. The strongest model fit was observed for estimated BW-dependent indices, especially CII, TI1, and TI2, which is expected because several indices include estimated BW or chest measurements in their calculation.

It is well established that selection criteria during marketing and breeding are determined by breeders and breeding associations. The health status and intended use of horses are also taken into consideration (Jönsson et al., 2014). Evaluating correlations between morphometric indices and ratios is important for determining genetic potential, describing breed standards, and supporting breed management and conservation programs aimed at increasing meat production (Younas et al., 2013). The correlation coefficients (r) among the 31 variables, including morphometric characteristics, estimated body weight, biometric indices, and ratios, are presented in the heatmap, with the corresponding values displayed within the cells (Fig. 2). Overall, most correlations in Algerian horses exceeded 0.6 ($P < 0.002$, $P < 0.0001$), indicating strong relationships and a high degree of homogeneity among linear measurements.

The results of the present study demonstrated a high degree of positive correlation among the examined horses. This may indicate that an increase in linear measurements is accompanied by a corresponding increase in estimated BW. This may be attributed to factors such as the availability of feed resources and the quality of management practices. The calculation of the correlation coefficients indicated that body weight exhibited a high correlation with ChC, compared with other measurements. This finding clearly suggests that chest circumference is the most reliable measurement for predicting the live weight of horses. The analysis of the correlation coefficients between the biometric indices and the ratios of the Algerian horse population revealed both negative and positive correlations ($P < 0.001$). Because ChC

is one of the input variables in the BW equation, its strong association with estimated BW is expected and should be interpreted as internal consistency within the estimation framework rather than as independent validation of ChC against directly measured live weight.

The present study indicates that morphometric analysis can support the description of animals with different body conformation indices. In this study, the results obtained are in close agreement with those reported by Guyo et al. (2024). The correlations observed for body conformation indices varied considerably, ranging from highly positive to markedly negative associations with the functional characteristics under investigation. Correlation results may help identify morphometric variables useful

for future selection or conservation programs, but they should not be considered sufficient evidence for advanced selection strategies without larger data sets, pedigree or molecular information, and direct functional assessment. This is achieved through the use of morphological variables employed in body conformation analysis (Ribeiro et al., 2024).

The present study has several limitations that should be acknowledged when interpreting the findings. First, the sample size was relatively modest ($n = 49$), which may have reduced statistical power and limited the generalizability of the results. Second, the study population was drawn from only two regions of Algeria; therefore, caution should be exercised when extrapolating these findings to the broader Arab-Barb horse population across the country. Third, BW was

Table 8. Morphometric index and ratio of the horses in Algeria according to area

Index/Ratio	Oran area (n = 34) (Mean \pm SD)	Bejaia area (n = 15) (Mean \pm SD)	P Value
BI	0.84 \pm 0.04	0.88 \pm 0.04	0.002
BR	1.06 \pm 0.05	0.99 \pm 0.05	< 0.001
CI1	3.23 \pm 0.29	3.12 \pm 0.57	0.473
DTI	0.111 \pm 0.006	0.116 \pm 0.007	0.012
RBI	0.94 \pm 0.04	1.01 \pm 0.05	< 0.001
TI1	109.83 \pm 7.65	108.86 \pm 14.63	0.811
TI2	186.32 \pm 12.98	184.67 \pm 24.83	0.811
TD	1.13 \pm 0.04	1.15 \pm 0.08	0.287
RTCI	0.125 \pm 0.007	0.133 \pm 0.007	< 0.001
FBH	1.01 \pm 0.02	1.00 \pm 0.02	0.102

Body index (BI); body ratio (BR); compact index 1 (CI1); dactyl thorax index (DTI); relative body index (RBI); tare index 1 (trot or gallop) (TI1); tare index 2 (walk) (TI2); thoracic development (TD); relative thickness of cannon bone index (RTCI); front-back height in (FBH).

Table 9. General linear model (GLM) analysis of the effect of sex, estimated body weight and region on morphometric index and ratios of Algerian horses

Index/ratio	Sex (P Value)	Estimated BW (P Value)	Region (P Value)	R ² (Model Fit)
BI	0.839	0.003	0.006	0.343
BR	0.296	0.714	< 0.001	0.362
CI1	0.526	< 0.001	0.844	0.593
DTI	0.003	0.08	0.017	0.367
RBI	0.323	0.678	< 0.001	0.371
TI1	0.379	< 0.001	0.339	0.530
TI2	0.379	< 0.001	0.339	0.530
TD	0.196	< 0.001	0.018	0.354
RTCI	0.063	0.18	< 0.001	0.294
FBH	0.057	0.092	0.175	0.157

Body ratio (BR); compact index 1 (CI1); dactyl thorax index (DTI); relative body index (RBI); tare index 1 (trot or gallop) (TI1); tare index 2 (walk) (TI2); thoracic development (TD); relative thickness of cannon bone index (RTCI); front-back height in (FBH).

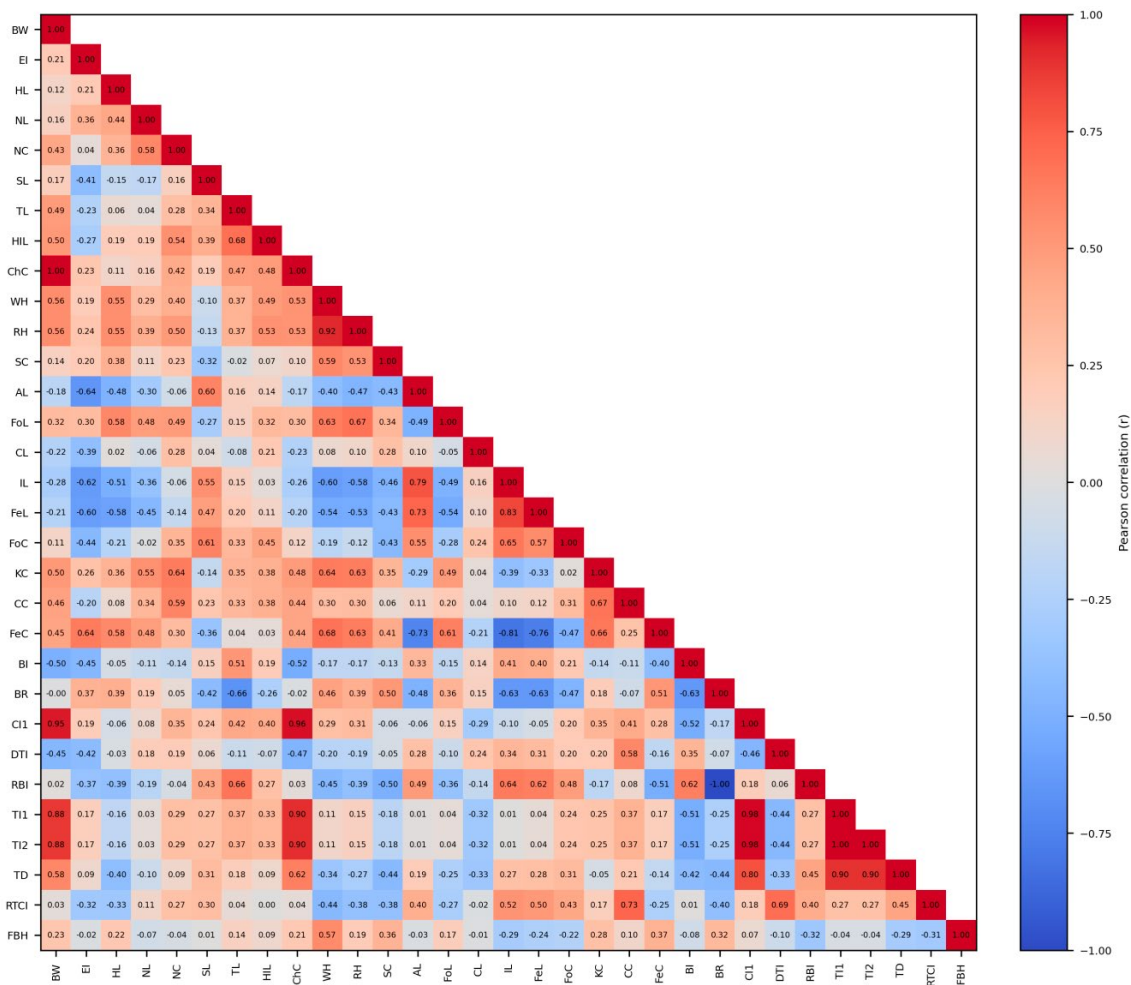


Fig.2. Phenotypic correlation coefficients (r) between 31 variables, including morphometric characteristics, estimated body weight, biometric indices, and ratios in Algerian horses

Estimated body weight (BW);eye to eye space (EI); head length (HL); neck length (NL); neck circumference (NC); shoulder length (SL); total length (TL); humero-iliac length (HIL); chest circumference (ChC); withers height (WH); rump height (RH); space under the chest (SC); arm length (AL); forearm length (FoL); cannon length (CL); ilium length (IL); femur length (FeL); forearm circumference (FoC); knee circumference (KC); cannon circumference (CC); fetlock circumference (FeC). Body index (BI); body ratio (BR); compact index 1 (CI1); dactyl thorax index (DTI); relative body index (RBI); tare index 1 (trot or gallop) (TI1); tare index 2 (walk) (TI2); thoracic development (TD); relative thickness of cannon bone index (RTCI); front-back height in (FBH).

estimated using an allometric equation originally developed for donkeys. Although this approach provides a practical alternative under field conditions where weighing facilities are unavailable, the equation has not yet been formally validated for Arab-Barb horses. Consequently, the derived BW values should be regarded as operational morphometric proxies rather than exact live-weight measurements. Accordingly, BW-based classifications and associated analyses should be interpreted as comparisons among estimated body mass categories rather than absolute weight-based assessments. Finally, athletic performance, endurance capacity, gait quality, and biomechanical function were not directly measured; consequently, functional interpretations based on morphometric indices should remain inferential.

Conclusion

The present study provides an updated morphometric profile of Algerian Arab-Barb horses from two northern regions and confirms a broadly homogeneous conformation, with measurable variation related to sex, estimated body weight, and geographic origin. The combination of linear measurements and body indices indicates a predominantly compact and breviline morphotype, offering a useful phenotypic reference for breed description, management, and conservation planning. Regional differences suggest that local environmental conditions, management systems, and population structure may contribute to conformational variability within Algerian Arab-Barb horses. These findings strengthen the morphometric basis for characterizing this national equine resource and highlight the importance of continued phenotypic monitoring combined with broader regional and molecular investigations.

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Beyond *Aeromonas hydrophila*: Isolation of *Aeromonas veronii* from Koi Carp (*Cyprinus carpio* var. *koi*) with Ulcerative Disease (Case Report)

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Keywords: koi carp, fish, bacteria, ulcerative syndrome, *Aeromonas veronii*.

Abstract: Koi carp (*Cyprinus carpio* var. *koi*) are globally popular ornamental fish, valued for their distinctive coloration and patterns. However, infectious disease outbreaks remain an important health concern, particularly bacterial infections associated with skin ulceration, systemic disease, and mortality. Motile aeromonad infections in freshwater fish, including koi carp, have traditionally been attributed mainly to *Aeromonas hydrophila*, while other species may have been underdiagnosed or overlooked. In this report, we describe the isolation of *Aeromonas veronii* from koi carp in a private collection that presented with lesions consistent with koi ulcer disease, along with the associated macroscopic findings.

Introduction

Aeromonas veronii is a Gram-negative, facultatively anaerobic bacterium widely distributed in freshwater environments and associated with infections in both aquatic animals and humans. In fish, it is considered an opportunistic pathogen that primarily affects immunocompromised hosts or those exposed to stressors such as poor water quality, temperature fluctuations, and overcrowding (Tasci et al., 2025). Infections caused by this organism have been reported in several fish species, including Nile tilapia (*Oreochromis niloticus*) (Aly et al., 2023), climbing perch (*Anabas testudineus*) (Ehsan et al., 2023), channel catfish (*Ictalurus punctatus*) (Hoai et al., 2019), among others.

Koi carp (*Cyprinus carpio* var. *koi*) are high-value ornamental freshwater fish selectively bred for their vivid coloration and distinctive patterns (Alavinezhad et al., 2021; Tasci et al., 2025). Originally developed in Japan, koi are now widely kept in garden ponds, private collections, and decorative aquatic systems worldwide. Despite their popularity and economic value, koi are susceptible to several infectious diseases, particularly bacterial infections affecting the skin and internal organs (Adamek et al., n.d.; Alavinezhad et al., 2021).

Koi ulcer disease (KUD) is an important condition in ornamental carp because it can cause severe skin ulceration, systemic infection, mortality, and economic losses in private collections and ornamental fish facilities. However, its epidemiology remains poorly defined, as many outbreaks occur in private ponds and are not systematically reported or investigated (Alavinezhad et al., 2021; Tasci et al., 2025). Disease emergence is commonly associated

with predisposing factors such as seasonal temperature changes, recent introduction of new fish, handling, transport, high stocking density, and poor water quality, which may compromise immune function and damage the skin barrier. KUD is characterized by cutaneous erosions and deep ulcerative lesions that may progress to systemic infection and death if left untreated (Adamek et al., n.d.; Alavinezhad et al., 2021). The disease has most frequently been associated with *Aeromonas* species, particularly *Aeromonas salmonicida* and *Aeromonas hydrophila* (Bai et al., 2023). However, other motile aeromonads, including *A. veronii*, may be underdiagnosed or overlooked in ulcerative syndromes of koi carp.

Therefore, the objective of this report is to describe the clinical, macroscopic, microscopic, bacteriological, and antimicrobial susceptibility findings associated with the isolation of *A. veronii* from koi carp belonging to a private collection presenting with lesions consistent with Koi ulcer disease. This case highlights the importance of accurate bacterial identification in ulcerative diseases of ornamental fish and contributes to the recognition of *A. veronii* as a potential pathogen associated with KUD.

Case Report

In the summer of 2025, a private owner submitted two deceased fish to the clinic following mortality events in a private outdoor artificial lake. The lake had an approximate volume of 20 000 L, was equipped with a UV filtration system, and its water temperature varied with ambient conditions. According to the owner, all the fish in the lake had exhibited similar macroscopic clinical signs. To date, only those two individuals have been found dead. The collection consisted of approximately 30 koi carp of varying sizes (100 g to 3 kg) and ages. Nearly all fish had

been acquired from suppliers in Germany, with the most recent acquisition occurring approximately six months before the onset of clinical signs and mortality. Clinical signs had been observed for approximately one week and progressively worsened despite corrective measures implemented by the owner, including cleaning of the tank, replacement of the filtration system, and disinfection of the water (salt and pergamation of potassium).

A post-mortem examination was subsequently performed. External examination revealed multifocal to diffuse scale loss associated with cutaneous ulcerations across the body surface (Figs. 1–2). Several ulcers (0.5–4 cm) were surrounded by hyperaemic margins and were most prominent in the cranial and caudal peduncle regions (Figs. 1–2). Erosive and frayed lesions of the caudal and pectoral fins were also observed. Additional findings included focal subcutaneous haemorrhages in the regions underlying the pectoral and pelvic fins, anal swelling, and moderate abdominal distension.

Internal examination revealed generalized congestion of the visceral organs. The gall bladder was markedly distended with bile, and the intestinal tract was hyperaemic, haemorrhagic, and inflamed. In one specimen, the gills appeared diffusely congested.

Samples obtained from skin ulcers and internal organs were inoculated onto Blood Agar, CNA, and MacConkey Agar plates (bioMérieux, France), and incubated aerobically and anaerobically at 35–37°C for 48 h. Bacterial isolates were identified using the VITEK® 2 system with the GN ID Card REF 21341 (bioMérieux, France). The isolates were identified as *Aeromonas veronii*. Automated antimicrobial susceptibility testing was conducted using the VITEK® 2 Compact system (bioMérieux, France) with the AST-GN98 card, following the manufacturer's instructions. Minimum inhibitory concentrations (MICs) were determined automatically and interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, CLSI VET01-S2). The antibiogram revealed

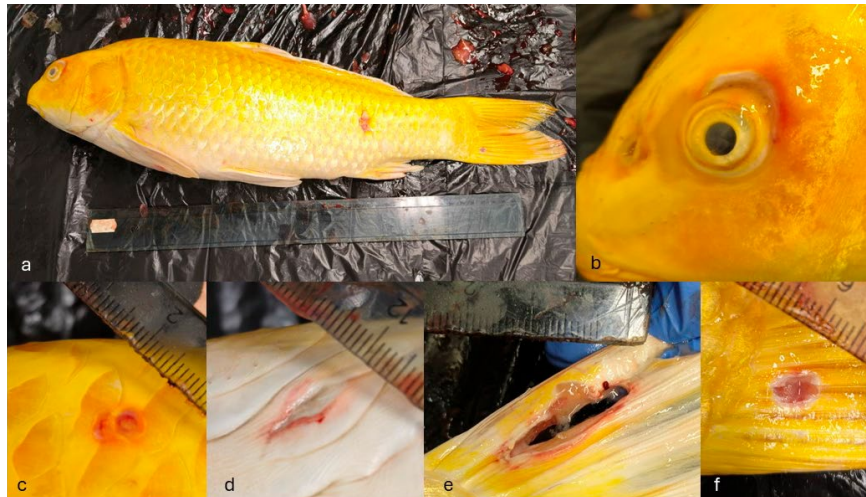


Fig. 1. Full body image from koi carp (*Cyprinus carpio* var. *koi*); b–f: ulcerative lesions.



Fig. 2. Full body image from koi carp (*Cyprinus carpio* var. *koi*); b–f: ulcerative lesions

resistance to tetracycline (MIC \geq 16.0 $\mu\text{g}/\text{mL}$) and susceptibility to amoxicillin-clavulanic acid (MIC = 8.0 $\mu\text{g}/\text{mL}$), gentamicin (MIC \leq 1.0 $\mu\text{g}/\text{mL}$), chloramphenicol (MIC \leq 2.0 $\mu\text{g}/\text{mL}$), trimethoprim-sulfamethoxazole (MIC \leq 20.0 $\mu\text{g}/\text{mL}$), enrofloxacin (MIC \leq 0.12 $\mu\text{g}/\text{mL}$), ceftiofur (MIC \leq 1.0 $\mu\text{g}/\text{mL}$), marbofloxacin (MIC \leq 0.5 $\mu\text{g}/\text{mL}$), and cefovecin (MIC \leq 0.5 $\mu\text{g}/\text{mL}$). Treatment was performed using daily enrofloxacin baths (Baytril[®] 2.5% oral solution, Bayer) for 5 h per day over a 7-day period. The fish were treated in a 5000 L tank at a concentration of 5 mg/L (Carpenter and Harms, 2022). At the end of the treatment course, the fish began to show clinical improvement, and no new lesions were observed.

Discussion

In this report, *A. veronii* was isolated from koi carp presenting with clinical and post-mortem signs consistent with KUD, including multifocal cutaneous ulcerations, scale loss, and systemic involvement. Although *A. salmonicida* and *A. hydrophila* are classically implicated in ulcerative and septicemic diseases of cyprinids, studies increasingly recognize *A. veronii* as a significant pathogenic agent in freshwater fish. *A. veronii* has been isolated from diverse fish species with clinical presentations involving dermal ulceration, haemorrhages, exophthalmos, and internal organ congestion similar to those seen in *A. hydrophila* infections (Aly et al., 2023).

The gross findings in the present cases mirror those documented for *A. veronii* infections in tilapia and carp species, where ulcerative syndrome and systemic pathology were prominent (Aly et al., 2023; Han et al., 2021; Wang et al., 2021). These manifestations also overlap extensively with signs reported in *A. hydrophila* infections, which include haemorrhagic skin lesions, tail and fin erosions, internal organ congestion, and high mortality under stress or poor environmental conditions (Bai et al., 2023; Shabana et al., 2025). Thus, from a clinical standpoint, *A. veronii*-associated ulcerative disease may be indistinguishable from *A. hydrophila*-mediated motile *Aeromonas* septicemia without bacteriological and molecular identification.

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Antimicrobial susceptibility varies widely among *Aeromonas* isolates, underlining the necessity of antimicrobial susceptibility testing for targeted therapy. The isolates in this case were resistant to tetracycline but susceptible to a range of agents, including enrofloxacin, gentamicin, and ceftiofur; this is consistent with susceptibility profiles in other reports where aeromonads showed variable resistance patterns but often remained susceptible to fluoroquinolones and aminoglycosides (Fadel et al., 2025; Han et al., 2021). The positive clinical response to enrofloxacin baths in the affected koi reinforces the importance of using *in vitro* susceptibility to guide treatment decisions. However, global concerns about antimicrobial resistance in aquatic pathogens call for the judicious use of antibiotics and exploration of alternative control strategies, such as vaccination or probiotics.

Environmental stressors, including suboptimal water quality and temperature fluctuations, are well-established risk factors for opportunistic *Aeromonas* infections in fish, lowering host defences and facilitating bacterial invasion. Although water parameters in this case were not extensively recorded, similar studies emphasize that poor water quality often precedes outbreaks of ulcerative and septicemic disease in tilapia and carp, further implicating environmental management as a cornerstone of disease prevention.

Conclusion

This case report identifies *Aeromonas veronii* as a bacterium associated with ulcerative disease and systemic lesions in koi carp (*Cyprinus carpio var. koi*). The clinical, macroscopic, and microscopic findings were consistent with Koi ulcer disease, while bacteriological culture and antimicrobial susceptibility testing supported the selection of enrofloxacin bath treatment. These findings highlight the importance of accurate bacterial identification in ulcerative diseases of ornamental fish, since motile aeromonad infections should not be attributed exclusively to *Aeromonas hydrophila*. Comprehensive diagnostic testing is therefore essential for targeted treatment and responsible antimicrobial use.

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Integrated Biomarker System for Assessing Reproductive Potential in Mares: A Review

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Keywords: mare fertility, equine reproduction, biomarkers, anti-Müllerian hormone, endometritis, cytokines, oxidative stress, lipidomics, reproductive biomarkers.

Abstract: Complex interactions among structural, endocrine, and molecular factors determine mare reproductive potential. This narrative review summarizes current research data on clinical, hormonal, and molecular–biochemical biomarkers associated with mare fertility and evaluates their potential use in reproductive assessment. Clinical assessment remains the foundation of reproductive evaluation and includes age, reproductive history, ultrasonography, cytology, bacteriology, and endometrial biopsy. Hormonal biomarkers such as anti-Müllerian hormone, estradiol, progesterone, and gonadotropins provide additional information on ovarian reserve, follicular activity, ovulation, and luteal competence. Increasing attention has also focused on molecular and biochemical biomarkers related to inflammation, oxidative stress, extracellular matrix remodeling, and embryonic–maternal communication. Despite growing interest in advanced biomarker approaches, many molecular indicators are not yet sufficiently validated for routine clinical use in mares. Small sample sizes, methodological heterogeneity, and a lack of standardized reference ranges often limit available studies. Therefore, interpretation of reproductive biomarkers should remain cautious and clinically contextualized. Available data suggest that integrating structural, endocrine, and molecular information may improve reproductive evaluation and enable more individualized management strategies in mares. However, prospective studies evaluating the predictive value and clinical utility of integrated biomarker models remain limited.

Introduction

Reproductive efficiency in mares is influenced by several physiological factors, including seasonal reproductive activity, prolonged gestation, age-related fertility decline, and the production of a single offspring per pregnancy (Cuervo-Arango et al., 2010; Aurich, 2011; Scarlet et al., 2025). Fertility declines progressively with advancing age. This decline is associated with cumulative ovarian and uterine alterations, including depletion of the follicular reserve, impaired oocyte competence, chronic endometrial degeneration, and increased embryonic loss. Even though routine reproductive examinations allow identification of many clinically evident abnormalities, fertility outcomes in mares often remain difficult to predict accurately.

Traditional reproductive assessment relies primarily on clinical evaluation, ultrasonography, uterine cytology and bacteriology, and endometrial biopsy (Crabtree and Pycock, 2020; Gonçalves et al., 2020). These methods remain essential in equine reproductive practice because they provide direct structural and functional information about ovarian activity and uterine health. Nevertheless, some mares

with apparently normal clinical findings continue to exhibit subfertility or repeated pregnancy failure, suggesting that clinically undetectable endocrine or molecular disturbances may contribute to impaired reproductive performance.

Consequently, researchers have increasingly explored biomarkers reflecting ovarian reserve, endocrine activity, inflammatory status, oxidative balance, and embryo–maternal communication. Anti-Müllerian hormone has emerged as one of the most widely studied endocrine biomarkers of ovarian reserve and assisted reproductive performance (Ball et al., 2008; Canesin et al., 2023; Fonte et al., 2024), while progesterone and estradiol remain important indicators of luteal function and follicular activity (Satué et al., 2021; Goudet et al., 2022). However, endocrine markers alone cannot fully explain the complex cellular and molecular mechanisms underlying fertility.

Advances in transcriptomics, proteomics, lipidomics, and biochemical profiling have identified molecular signatures linked to uterine inflammation, oxidative stress, extracellular matrix remodeling, and early embryo–maternal signaling (Canisso et al., 2020; Ibrahim et al., 2021; Lawson et al., 2022; Silva-Álvarez et al., 2025). These approaches have the

potential to identify reproductive disturbances before overt clinical pathology becomes apparent. However, many proposed molecular biomarkers remain insufficiently validated for routine clinical use because small sample sizes, methodological heterogeneity, and lack of standardized reference ranges often limit available studies.

Because reproductive competence depends on interactions among multiple physiological systems, interpretation based on a single biomarker category may provide only partial information. Integration of clinical, endocrine, and molecular findings has therefore been proposed as a potential strategy to improve reproductive assessment in mares (Papaas et al., 2021; Fouché et al., 2022). Nevertheless, evidence supporting integrated biomarker-based assessment in mares is still limited and fragmented.

This review critically evaluates the current data on clinical, hormonal, and molecular-biochemical biomarkers related to the reproductive potential of mares. Even if these biomarker groups have been described separately in previous studies, their practical integration remains poorly defined. In addition to summarizing available data, this narrative review examines the relative relevance of individual biomarkers and proposes a clinically oriented model for their interpretation in reproductive decision-making.

Literature Search Strategy

This structured narrative review employed a transparent literature search strategy to identify relevant studies on biomarkers associated with reproductive potential in mares. Literature searches were performed in PubMed, Scopus, and Web of Science databases using combinations of the following keywords: *mare fertility, equine reproduction, biomarkers, anti-Müllerian hormone, endometritis, cytokines, oxidative stress, lipidomics, and reproductive biomarkers.*

A total of 312 records were identified across all databases. After removing 74 duplicate records, 238 publications remained for title and abstract screening. During the initial screening phase, 151 records were excluded because they were unrelated to reproductive biomarkers in mares, focused on other species, or did not address fertility assessment. The full texts of 87 articles were assessed for eligibility.

Studies were excluded if they: were conference abstracts, editorials, book chapters, or non-peer-reviewed publications; focused exclusively on other equine species or unrelated reproductive conditions; lacked methodological transparency; or did not provide direct evidence relevant to fertility assessment in mares.

After full-text review of 87 articles, 37 studies were rejected because they did not meet the eligibility criteria. Finally, 50 publications were selected for qualitative synthesis because they provided direct

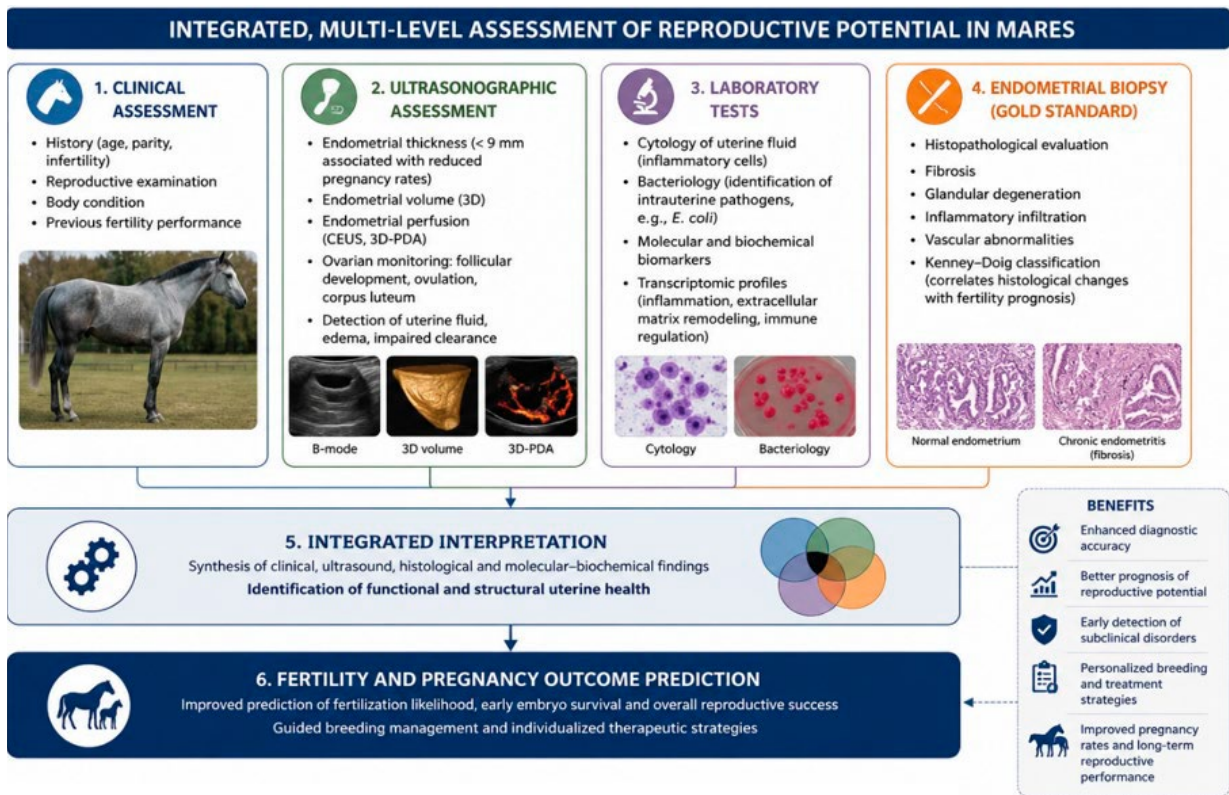


Fig. 1. Schematic representation of the integrated multi-level clinical assessment of reproductive potential in mares (adapted from Katila, 2016; Ferreira et al., 2015; Crabtree and Pycoc, 2020; Claes et al., 2017; Gonçalves et al., 2020; Fawcett et al., 2021; Morris et al., 2020; Ferreira-Dias et al., 2024; Ishak et al., 2019.) Abbreviations: CEUS, contrast-enhanced ultrasonography; 3D-PDA, three-dimensional power Doppler angiography.

evidence on the use of clinical, hormonal, molecular, and biochemical biomarkers to assess reproductive potential and fertility in mares. The final selection of studies was based on their relevance to the review objectives and the authors' critical appraisal of the available evidence.

Clinical Biomarkers

Clinical biomarkers remain the cornerstone of reproductive evaluation in mares, providing essential information about the structural and functional integrity and reproductive potential of the organism. These indicators – age, reproductive history, cytology of uterine fluid, bacteriology, endometrial biopsy, and ultrasound – capture macroscopic and physiological changes that directly affect fertility and remain irreplaceable in normal reproductive practices (Katila, 2016; Crabtree and Pycock, 2020; Cortés-Vidauri et al., 2018).

Mare age is one of the main determinants of reproductive potential, reflecting changes in the ovarian reserve, uterus, and endocrine system, because fertility gradually declines with age (Claes et al., 2017; Gonçalves et al., 2020; Fawcett et al., 2021). Older mares exhibit reduced antral follicle counts, slower follicular growth, prolonged interovulatory intervals, and decreased follicular maturation efficiency, along with reduced expression of epidermal growth factor receptor (EGFR), vascular endothelial growth factor

(VEGF), luteinizing hormone receptor (LHR), and the Ki67 marker in follicular cells. Ki67 is a classic marker of cell proliferation that indicates granulosa cell activity and follicular growth rate (Campos-Chillon et al., 2015). These changes, combined with age-related mitochondrial dysfunction and increased chromosomal abnormalities in follicles, reduce embryonic viability and increase the risk of early embryo loss (Hendriks et al., 2015; Rizzo et al., 2019). Age-related uterine degeneration, including fibrosis, glandular atrophy, and increased risk of endometriosis and collagen type III (COL3), further reduces the likelihood of fertilization (Ferreira et al., 2015; Sikora et al., 2016; Schöniger and Schoon, 2020; Morris et al., 2020).

This schematic in Fig. 2 illustrates the age-associated decline in reproductive potential in mares and the interconnected ovarian, uterine, and cellular mechanisms contributing to reduced fertility. An optimal ovarian reserve, high oocyte quality, and minimal fibrotic changes within ovarian and endometrial tissues characterize young mares. During the transition to middle age, declining anti-Müllerian hormone (AMH) concentrations and antral follicle count (AFC) are accompanied by increasing oxidative stress, resulting in reduced embryo competence and lower developmental potential. In aged mares, ovarian reserve is markedly diminished, while chronic inflammation, fibrosis, endometriosis, and

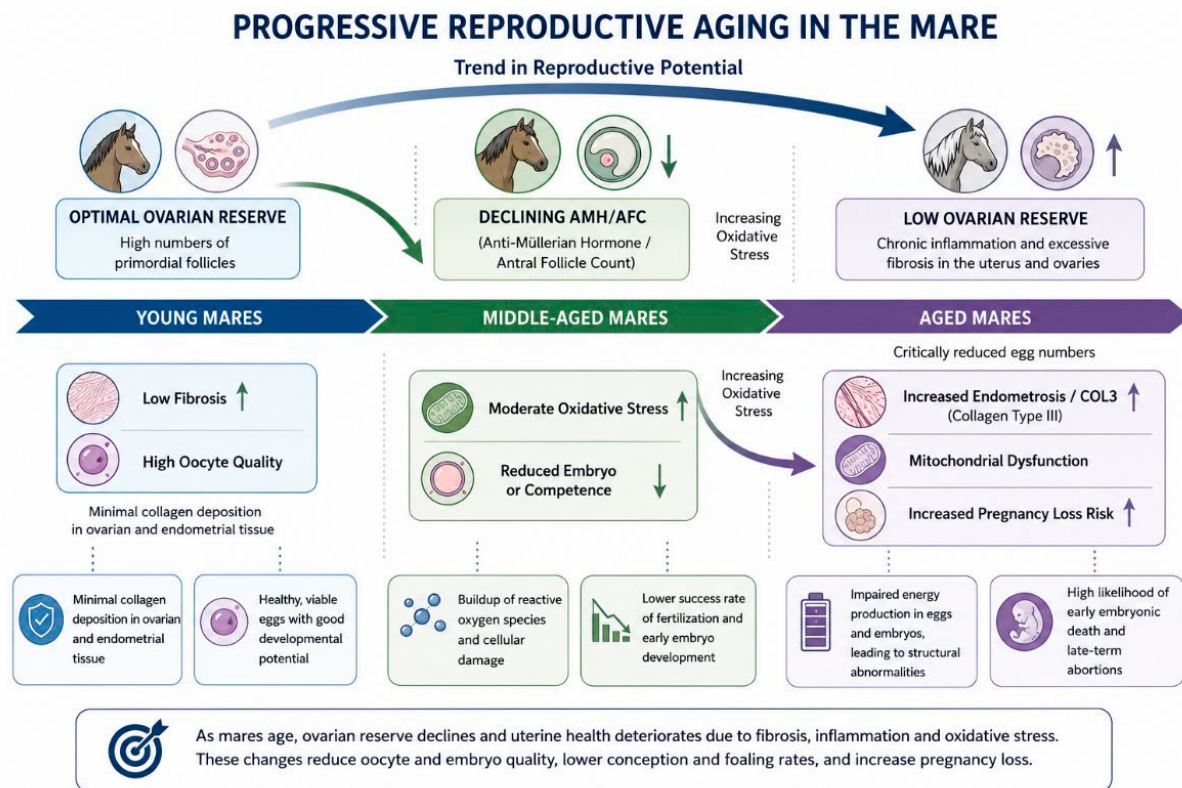


Fig. 2. Progressive reproductive aging and its impact on fertility outcomes in mares (adapted from Claes et al., 2017; Gonçalves et al., 2020; Fawcett et al., 2021; Campos-Chillon et al., 2015; Hendriks et al., 2015; Rizzo et al., 2019; Morris et al., 2020; Cortés-Vidauri et al., 2018). Abbreviations: AMH, anti-Müllerian hormone; AFC, antral follicle count; COL3, collagen type III.

increased deposition of type III collagen (COL3) further compromise reproductive function. Age-related mitochondrial dysfunction and oxidative damage impair oocyte and embryo quality, increasing the likelihood of early embryonic loss and pregnancy failure. Collectively, these progressive alterations reduce fertilization success, embryo viability, and overall reproductive performance in mares.

Reproductive history is an important clinical indicator of reproductive performance, reflecting previous reproductive capacity and providing retrospective evidence of general health status and reproductive system competence. Mares with a history of successful foaling tend to have higher rates of fertilization and fetal delivery, whereas mares that have not been fertilized or have had previous problems often have underlying uterine pathology, impaired clearance mechanisms, or endocrine dysfunction (Katila, 2016; Cecchini et al., 2023). Recurrent endometritis, accumulation of intrauterine fluid, or early embryo loss are particularly informative indicators of uterine dysfunction. Subclinical endometritis is frequently observed in intensively managed breeding mares and is especially common in older mares with slowed natural uterine clearance (Ferreira et al., 2015; Morris et al., 2020; Ferreira-Dias et al., 2024).

In such cases, cytology and bacteriology of the uterine fluid are necessary for diagnosing endometritis, enabling identification of inflammatory cells and intrauterine pathogens. Recent research has advanced current knowledge and identified an important subset of mares with abundant *E. coli* growth, yet important knowledge gaps remain regarding the etiologies that lead to poorer fertility outcomes (Fehin et al., 2025). Endometrial biopsy remains the gold standard for structural assessment of uterine health, especially in older mares with chronic endometritis (Silva Rua et al., 2018). Histopathological evaluation of uterine mucosal samples allows detection of fibrosis, glandular degeneration, inflammatory infiltration, and vascular abnormalities that impair uterine receptivity and fertility (Buchovska et al., 2016; Schöniger and Schoon, 2020). Chronic inflammation and fibrosis of the endometrium are closely related to fertilization and increased early embryo loss. The Kenney-Doig classification system correlates the intensity of histological changes in the uterine mucosa with the likelihood of fertilization, so biopsy remains a powerful prognostic tool for reproductive potential. Recent transcriptomic studies also show that mares with endometrosis exhibit altered expression of genes involved in inflammation, extracellular matrix remodeling, and immune regulation (Drzewiecka et al., 2025).

The uterine endometrium provides the critical interface for embryo implantation and pregnancy establishment. Endometrial receptivity – the capacity of the endometrium to accept an embryo – depends on precisely timed endocrine signaling, vascular

development, and immune regulation. While subjective ultrasonographic morphology assessment has been the traditional approach to endometrial evaluation in mares, emerging evidence demonstrates that quantitative ultrasound parameters enhance predictive accuracy for pregnancy outcomes.

Endometrial thickness represents one of the most commonly assessed parameters, with studies demonstrating that thickness below 9 mm is significantly associated with reduced implantation rates. More sophisticated ultrasound assessments, including three-dimensional (3D) imaging to measure endometrial volume and subendometrial blood flow, provide additional predictive information. Advanced ultrasound technologies such as contrast-enhanced ultrasonography (CEUS) and three-dimensional power Doppler angiography (3D-PDA) enable assessment of endometrial perfusion and microvascular function (Ferreira-Dias et al., 2024).

Transrectal ultrasound is a valuable tool for monitoring follicular development, ovulation timing, and corpus luteum formation, all of which are essential for optimizing breeding management (Ortega-Ferresola et al., 2022; Panzani et al., 2024; Khan et al., 2025). Additionally, ultrasound can detect abnormalities such as uterine fluid, abnormal endometrial edema, or impaired uterine clearance, findings that can directly predict the chances of fertilization. Seasonal variations in ovarian activity and patterns of follicular waves further highlight the importance of ultrasound in mare breeding. It is the primary method for early pregnancy diagnosis and monitoring embryonic development (Ishak et al., 2019). Despite its established clinical relevance, the use of clinical biomarkers may be limited by the inability to detect early or subclinical dysfunction. Most available evidence is derived from observational and retrospective studies, which can introduce biases related to mare selection, management conditions, and diagnostic criteria. Furthermore, clinical data often reflect late-stage pathology rather than early functional disorders, underscoring the need for additional hormonal and molecular-biochemical biomarkers.

For older mares with reproductive issues, the diagnostic and prognostic value of clinical tests is enhanced when findings are interpreted alongside hormonal and molecular-biochemical biomarkers, according to an integrated, multi-level assessment algorithm for reproductive potential.

Hormonal Biomarkers

Hormonal biomarkers are valuable indicators of reproductive function because they reflect activity within the hypothalamic–pituitary–gonadal (HPG) axis and provide information on ovarian reserve, follicular development, ovulation, and luteal function. Because endocrine regulation governs both the estrous cycle and the establishment of early

pregnancy, deviations in hormonal profiles serve as sensitive indicators of reproductive dysfunction. Unlike structural evaluation alone, hormonal biomarkers reflect dynamic physiological processes that vary throughout the reproductive cycle (Aurich, 2011; Ishak et al., 2019).

Anti-Müllerian hormone (AMH) is currently considered the most informative endocrine marker of ovarian reserve in mares. Circulating AMH concentrations correlate with antral follicle count, oocyte yield, and reproductive lifespan. Consequently, AMH is widely used to estimate the likelihood of successful oocyte recovery and embryo production in assisted reproductive programs (Ball et al., 2008; Fonte et al., 2024; Canesin et al., 2023). AMH is also used to select donor mares for OPU/ICSI programs, as higher AMH concentrations are associated with greater oocyte recovery and embryo production efficiency (Papaas et al., 2021). However, AMH does not directly predict pregnancy outcome and exhibits interindividual variability; it remains the strongest available indicator of granulosa cell activity and follicular pool size. Age-related reductions in AMH and altered antioxidant status further reflect declining ovarian reserve in older mares (Aboelmaaty et al., 2024).

Estradiol (E2), produced by granulosa cells of developing follicles, reflects follicular competence and readiness for ovulation. Increasing estradiol concentrations during the follicular phase indicate granulosa cell proliferation and aromatase activity, while reduced estradiol levels – particularly in aged or subfertile mares – are associated with impaired follicular development and decreased fertility (Goudet et al., 2022; Satué et al., 2021; Gonçalves et al., 2020). Estradiol also modulates uterine receptivity and immune responses, thereby influencing susceptibility to endometritis and potentially affecting embryo survival (Fumuso et al., 2000; Klein et al., 2010).

Progesterone (P4), secreted by the corpus luteum, is essential for establishing and maintaining pregnancy. It supports endometrial secretory transformation, suppresses uterine contractility, and promotes embryo development. Inadequate progesterone production has been linked to an increased risk of early embryonic loss, highlighting its clinical relevance in assessing luteal function (Satué et al., 2021; Goudet et al., 2022). Progesterone metabolites, including allopregnanolone, offer additional insight into gestational endocrine activity, although their use remains primarily research-based (Satué et al., 2023; Veronesi et al., 2025).

Gonadotropins, including folliclestimulating hormone (FSH) and luteinizing hormone (LH), regulate follicular recruitment, maturation, and ovulation. Their pulsatile secretion reflects hypothalamic and pituitary function and is influenced by photoperiod, metabolic status, and environmental

cues (Aurich, 2011; Donadeu et al., 2014; CortésVidauri et al., 2018). Seasonal reproductive transitions are accompanied by significant changes in gonadotropin secretion and ovarian responsiveness (Ishak et al., 2019). Interpretation is complicated by their pulsatile secretion patterns and the limited diagnostic value of individual measurements.

Additional steroid and stress-related hormones may offer complementary information about endocrine aging, metabolic status, and systemic stress responses. Although changes in these hormones have been linked to reproductive dysfunction, their diagnostic value is less established than that of AMH, progesterone, or estradiol (Falomo et al., 2020; Satué et al., 2023; Veronesi et al., 2025). Lipidomic and steroidomic approaches may identify additional metabolic indicators linked to reproductive performance (Lawson et al., 2022).

Interpretation of hormonal biomarkers is complicated by temporal variability, as most endocrine parameters strongly depend on cycle stage, season, and sampling time. Many studies rely on single-time-point measurements, which may not accurately reflect dynamic endocrine function. Additionally, interindividual variability and the lack of standardized reference ranges limit their predictive value across different mare populations.

Endocrine biomarkers provide information that structural assessment alone cannot offer. When integrated with clinical and molecular-biochemical biomarkers, hormonal profiles enable more precise assessment of reproductive potential and improved reproductive management strategies.

A summary of hormonal biomarkers associated with reproductive potential in mares is provided in Table 1.

Hormonal biomarkers reflect functional endocrine activity of the hypothalamic-pituitary-gonadal axis and provide essential information on ovarian reserve, follicular competence, ovulatory function, and luteal activity. Anti-Müllerian hormone is the most reliable indicator of ovarian reserve, while progesterone and estradiol reflect luteal competence and follicular activity. Gonadotropins and additional steroid hormones provide complementary insight into endocrine regulation and reproductive potential.

The integration of hormonal biomarkers within the hypothalamic-pituitary-gonadal axis and their relevance for reproductive potential is illustrated in Fig. 3.

Hormonal biomarkers reflect endocrine regulation of follicular development, ovulation, luteal function, and uterine receptivity. Anti-Müllerian hormone reflects ovarian reserve, estradiol indicates follicular competence, and progesterone reflects luteal function and pregnancy support. Gonadotropins regulate ovarian activity, while additional steroid hormones provide complementary information on systemic endocrine regulation and reproductive status.

Table 1. Hormonal biomarkers associated with reproductive potential in mares

Hormone	Clinical Interpretation	Prognostic Value	Limitations	Clinical Application Level	Key References
AntiMüllerian hormone (AMH)	Indicates ovarian reserve, predicts oocyte yield and response to ART (OPU-ICSI)	High	Does not directly predict pregnancy outcome; inter-individual variability	Widely used, validated	Ball et al., 2008; Fonte et al., 2024; Papaas et al., 2021
Progesterone (P4)	Assesses luteal function and pregnancy maintenance	High	Single measurements may not reflect dynamic luteal function	Routine clinical use	Ginther, 2008; Goudet et al., 2022; Satué et al., 2021
Estradiol (E2)	Indicates follicular competence and ovulatory readiness	Moderate-High	Strong cyclestage dependency	Routine clinical use	Goudet et al., 2022; Aboelmaaty et al., 2024; Satué et al., 2021
Folliclestimulating hormone (FSH)	Reflects ovarian responsiveness and follicular recruitment	Moderate	Pulsatile secretion complicates interpretation	Limited clinical use	Aurich, 2011; Cortés-Vidauri et al., 2018
Luteinizing hormone (LH)	Indicates ovulatory function and reproductive cyclicity	Moderate	Short surge duration limits the diagnostic window	Limited clinical use	Aurich, 2011; Cortés-Vidauri et al., 2018
Testosterone	Reflects ovarian steroidogenic activity	Moderate	Age and cycle-dependent variation	Emerging clinical relevance	Donadeu et al., 2014; Satué et al., 2023
Dehydroepiandrosterone (DHEA)	Indicates endocrine aging and reproductive endocrine status	Emerging	Limited clinical reference ranges	Research stage	Satué et al., 2023; Veronesi et al., 2025
Cortisol	Indicates stress-related reproductive suppression	Emerging	Influenced by environmental and physiological stressors	Research stage	Veronesi et al., 2025
Progesterone metabolites (e.g., allopregnanolone)	Indicate pregnancy progression and luteal function	Emerging	Primarily research application	Research stage	Veronesi et al., 2025

Molecular and Biochemical Biomarkers

Molecular and biochemical biomarkers enable the assessment of cellular and molecular processes that routine clinical tests cannot directly measure. These biomarkers can detect early cellular changes before clinical signs of reproductive disease appear. However, their clinical application remains limited. Many published studies involve relatively small populations, different analytical methods, and non-standardized sampling procedures, making direct comparison of results difficult. For this reason, most molecular biomarkers should currently be considered promising research tools rather than established diagnostic markers (Morris et al., 2020; Falomo et al., 2020; Lawson et al., 2022; Silva-Álvarez et al., 2025).

Even though most molecular biomarkers are still in the research stage, they may eventually complement routine reproductive assessment by improving our understanding of the biological mechanisms involved

in fertility. The main groups of molecular and biochemical biomarkers relevant to the reproductive potential of mares are summarized in Table 2.

Inflammatory biomarkers are among the most extensively studied molecular indicators of uterine dysfunction in mares. Subclinical endometritis is associated with increased production of inflammatory mediators like prostaglandin E₂ (PGE₂), 6ketoPGF₁α, and leukotriene C₄, which disrupt immune homeostasis and impair uterine function (Siemieniuch et al., 2017). Older mares exhibit heightened inflammatory responses, contributing to increased susceptibility to persistent breeding-induced endometritis (Morris et al., 2020; FerreiraDias et al., 2024). Altered cytokine expression has been linked to differences in uterine immune responsiveness between resistant and susceptible mares. This suggests potential value in identifying mares predisposed to reproductive dysfunction (Fumuso et al., 2000; Klein

et al., 2010). Neutrophil extracellular traps (NETs) further contribute to endometrial inflammation and tissue damage, exacerbating reproductive dysfunction (Rebordão et al., 2018; Rebordão et al., 2021).

Oxidative stress has received increasing attention because excessive levels of reactive oxygen species can impair oocyte quality, embryo development, and uterine receptivity. Several markers of oxidative stress have been associated with impaired reproductive

function and reduced uterine function (Agarwal et al., 2014; Boni et al., 2022). Oxidative dysregulation is particularly relevant in older mares, where reduced antioxidant capacity and increased levels of oxidative metabolites correlate with reduced ovarian reserve and altered AMH dynamics (Aboelmaaty et al., 2024). Oxidative stress has also been associated with reproductive disorders and impaired uterine function in mares (Falomo et al., 2020).

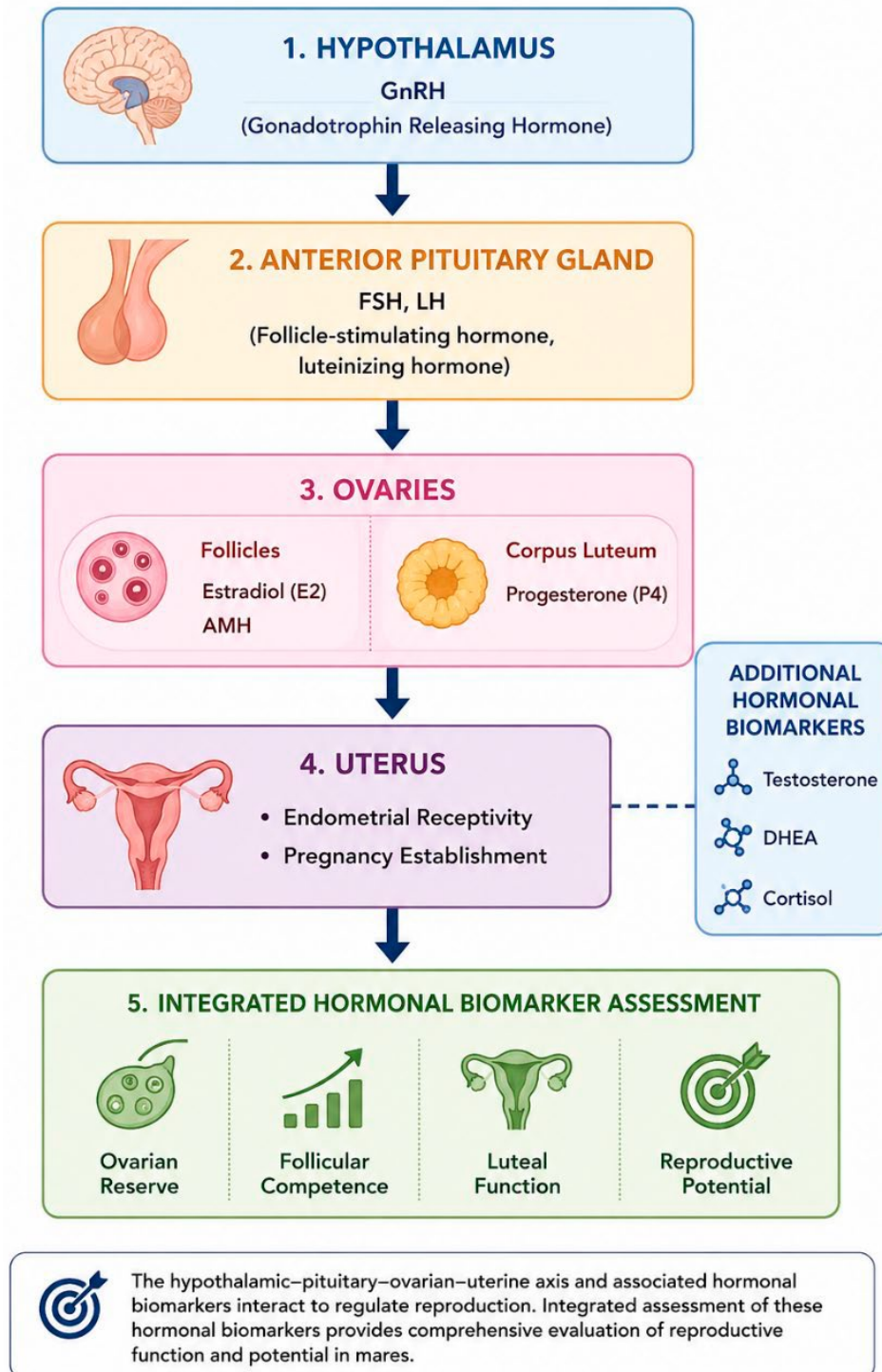


Fig. 3. Integration of hormonal biomarkers within the hypothalamic–pituitary–gonadal (HPG) axis and their role in determining reproductive potential in mares (adapted from Ball et al., 2008; Ginther, 2008; Aurich, 2011; Veronesi et al., 2025).

Table 2. Molecular and biochemical biomarkers associated with reproductive potential in mares

Biomarker Group	Clinical Interpretation Level	Clinical Application Level	Key References
Cytokines	Indicators of uterine inflammation and immune competence	Emerging and transitioning to clinical use	Fumuso et al., 2000; Klein et al., 2010
Acute-phase proteins	Biomarkers of uterine inflammation and reproductive pathology	Limited clinical use	Falomo et al., 2020; Morris et al., 2020
Oxidative stress biomarkers	Indicators of endometrial dysfunction and reproductive stress	Research only	Agarwal et al., 2012; Boni et al., 2022
MicroRNAs	Biomarkers of endometritis and reproductive dysfunction	Research only	Ibrahim et al., 2021
Proteomic biomarkers	Biomarkers of early pregnancy and reproductive status	Research only	Ortega-Ferresola et al., 2022
Extracellular matrix biomarkers	Indicators of endometrial fibrosis and reduced fertility	Research-clinical transition	Ferreira Dias et al., 2024
Lipidomic biomarkers	Reflect uterine inflammation and reproductive function	Research only	Ibrahim et al., 2021; Lawson et al., 2022

Extracellular matrix (ECM) remodeling biomarkers capture structural and functional changes in the endometrium. Altered expression of extracellular matrix remodeling factors has been associated with fibrosis and decreased uterine receptors (Schöniger and Schoon, 2020; Ferreira-Dias et al., 2024). ECM remodeling is closely associated with chronic endometritis and degenerative uterine changes, including endometriosis, which is a major factor in reduced fertility in older mares. Proteolytic enzymes and NET-associated proteases further contribute to ECM degradation and dysregulated prostaglandin synthesis (Rebordão et al., 2018; Rebordão et al., 2021).

Transcriptomic and proteomic approaches have expanded current understanding of embryo-maternal communication and uterine function. High-throughput analyses have identified molecular signatures linked to embryo viability and uterine receptivity (Morris et al., 2020; Silva-Álvarez et al., 2025). Transcriptomic studies have also demonstrated age-related alterations in myometrial gene expression and pathways associated with inflammation, ECM remodeling, and immune regulation (Drzewiecka et al., 2025). Circulating microRNAs are being studied as potential non-invasive biomarkers for uterine dysfunction (Ibrahim et al., 2021).

Lipidomic and steroidomic analyses are a relatively new area of equine reproductive research. Lipid mediators, steroid metabolites, and bioactive lipids reflect inflammatory status, oxidative balance, and steroidogenic activity, offering additional mechanistic insight into reproductive competence (Falomo et al., 2020; Lawson et al., 2022). These biomarkers may help identify mares with subtle metabolic or inflammatory disturbances that affect fertility but remain undetected by conventional endocrine or clinical assessment.

Overall, molecular and biochemical biomarkers provide valuable information about the cellular mechanisms underlying reproductive function. Their

main value lies in identifying subtle inflammatory, oxidative, or fibrotic changes that may otherwise remain undetected. Nevertheless, broader clinical application will require larger validation studies, methodological harmonization, and clearer reference ranges.

Integrated Biomarker-Based Reproductive Potential Assessment System

Reproductive performance in mares is influenced by the interaction of structural, endocrine, immunological, and molecular processes. Each biomarker category reflects a distinct aspect of reproductive physiology and provides additional information for assessing fertility. The assessment system proposed in this review integrates clinical, hormonal, and molecular-biochemical indicators into a conceptual multi-level approach to reproductive assessment (Papaas et al., 2021; Fouché et al., 2022).

The proposed system is based on clinical indicators that provide basic information about the integrity of the reproductive system, ovarian activity, and reproductive function history. Age, reproductive history, ultrasonographic findings, and endometrial biopsy collectively establish the structural and functional context within which endocrine and molecular processes operate (Katila, 2016; Crabtree and Pycock, 2020).

Endocrine biomarkers play a functional component in the system, characterizing ovarian activity and reproductive cycles. Anti-Müllerian hormone reflects granulosa cell activity and follicular pool size (Ball et al., 2008; Fonte et al., 2024; Canesin et al., 2023), estradiol indicates follicular maturation, and progesterone represents luteal function and early pregnancy maintenance (Satué et al., 2023; Goudet et al., 2022). Gonadotropins and other steroid hormones further characterize hypothalamic-pituitary-gonadal axis activity and seasonal reproductive transitions (Aurich, 2011; CortésVidauri et al., 2018).

Molecular and biochemical markers provide mechanistic information about inflammatory, oxidative, and extracellular matrix processes that may affect fertility. Cytokines, oxidative stress indicators, ECM remodeling factors, lipid mediators, and transcriptomic signatures may help identify biological alterations associated with subclinical reproductive dysfunction (Morris et al., 2020; Ibrahim et al., 2021; Lawson et al., 2022; Silva-Álvarez et al., 2025). Integration of these biomarker categories may provide a more comprehensive assessment of reproductive potential than evaluation of individual markers alone.

However, not all biomarker categories contribute equally to clinical decision-making. The authors propose the following categorization based on the

current level of validation, reproducibility, and clinical applicability reported in the literature. Step 1 (high clinical relevance) includes age, reproductive history, ultrasonographic findings, endometrial biopsy, anti-Müllerian hormone, progesterone, and estradiol, which are supported by consistent evidence and widely used in clinical decision-making. Step 2 (moderate relevance) comprises gonadotropins, uterine cytology and bacteriology, and selected acute-phase proteins, which provide supportive but context-dependent information. Step 3 (emerging or research-stage biomarkers) includes cytokines, oxidative stress markers, microRNAs, and proteomic and lipidomic biomarkers, which offer mechanistic insight but currently lack standardized validation and

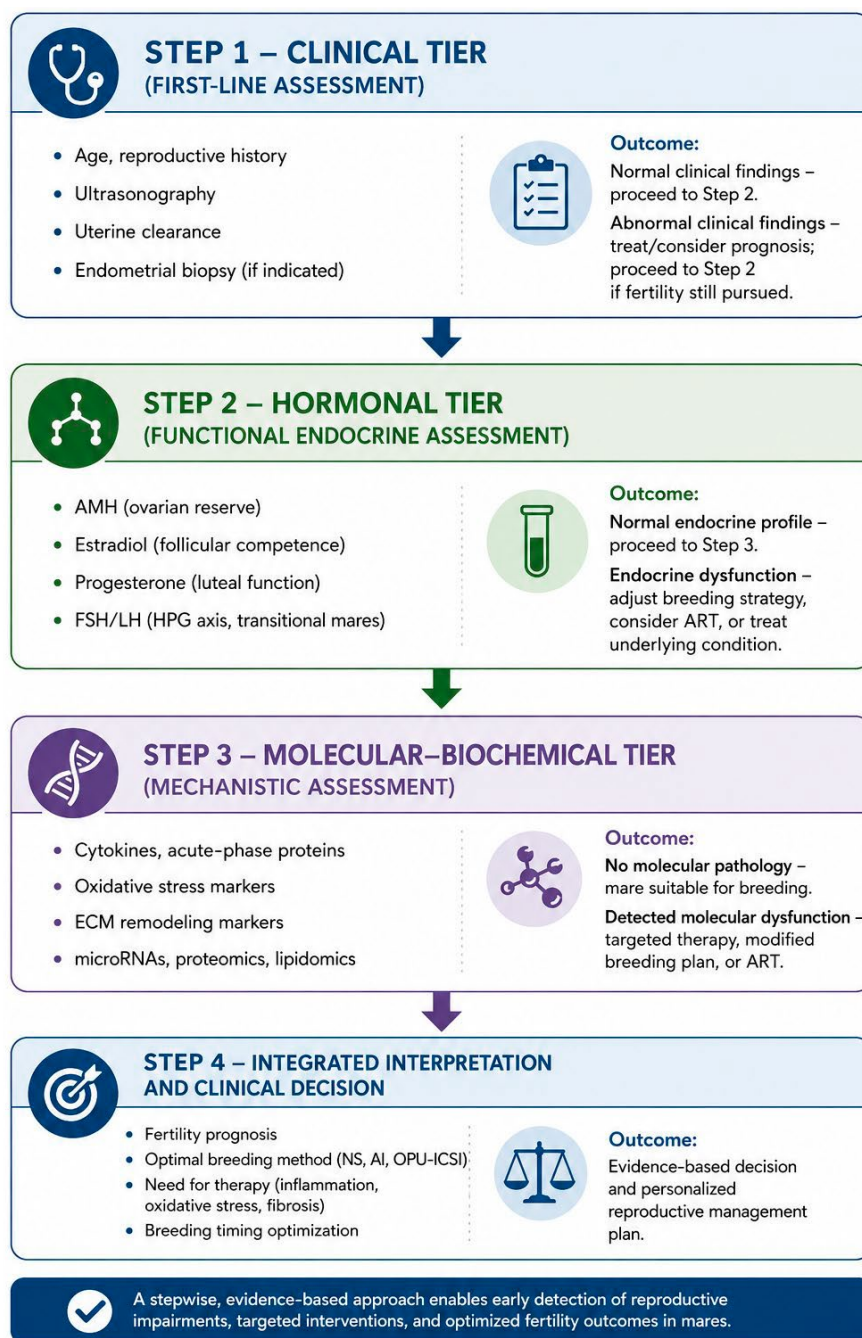


Fig. 4. Clinical decision algorithm for assessing reproductive potential in mares

routine clinical applicability.

This prioritization highlights that, despite increasing interest in molecular biomarkers, clinical and selected hormonal indicators currently provide the most robust and actionable information for fertility assessment in mares (Fig. 4).

This three-step decision algorithm guides clinicians through a series of assessments to identify structural abnormalities, endocrine dysfunction, and molecular pathology that may affect the reproductive potential of mares.

Evidence supporting integrated biomarker assessment is increasing; however, the relative contribution of individual biomarker groups to fertility prediction remains unclear. Clinical assessment and selected endocrine biomarkers provide the most reproducible and clinically applicable information, while many molecular biomarkers remain primarily research tools that require further validation before routine implementation can be recommended (Fouché et al., 2022).

Discussion

The assessment of reproductive potential in mares remains challenging because fertility is influenced by numerous interacting physiological, anatomical, endocrine, and environmental factors (Aurich, 2011; Gonçalves et al., 2020). Although individual biomarkers have been associated with specific reproductive outcomes, relatively few studies have evaluated how different biomarker categories perform when interpreted together (Papaas et al., 2021; Fouché

et al., 2022). Therefore, the framework proposed in this review should be viewed as a synthesis of currently available evidence rather than a validated predictive model.

Clinical examination continues to provide the foundation for reproductive evaluation. Parameters such as mare age, reproductive history, ultrasonographic findings, uterine cytology, bacteriology, and endometrial biopsy offer direct information regarding reproductive tract health and have established diagnostic relevance in equine practice. Also, understanding the normal events of foaling, causes of dystocia, and clinical outcomes is important for equine practitioners. (Katila, 2016; McCue and Ferris, 2011; Crabtree and Pycoc, 2020). Age-related reproductive decline, impaired uterine clearance, chronic inflammation, and endometrial fibrosis are consistently associated with reduced fertility and increased pregnancy loss (Ferreira et al., 2015; Claes et al., 2017; Morris et al., 2020; Ferreira-Dias et al., 2024). However, mares with apparently normal clinical findings may still experience subfertility, indicating that structural assessment alone does not always capture the biological mechanisms affecting reproductive success (Gonçalves et al., 2020; Fawcett et al., 2021).

Endocrine biomarkers provide complementary information by reflecting functional activity within the hypothalamic–pituitary–gonadal axis (Aurich, 2011; Ishak et al., 2019). Among currently available hormonal indicators, anti-Müllerian hormone appears to be the most informative marker of ovarian

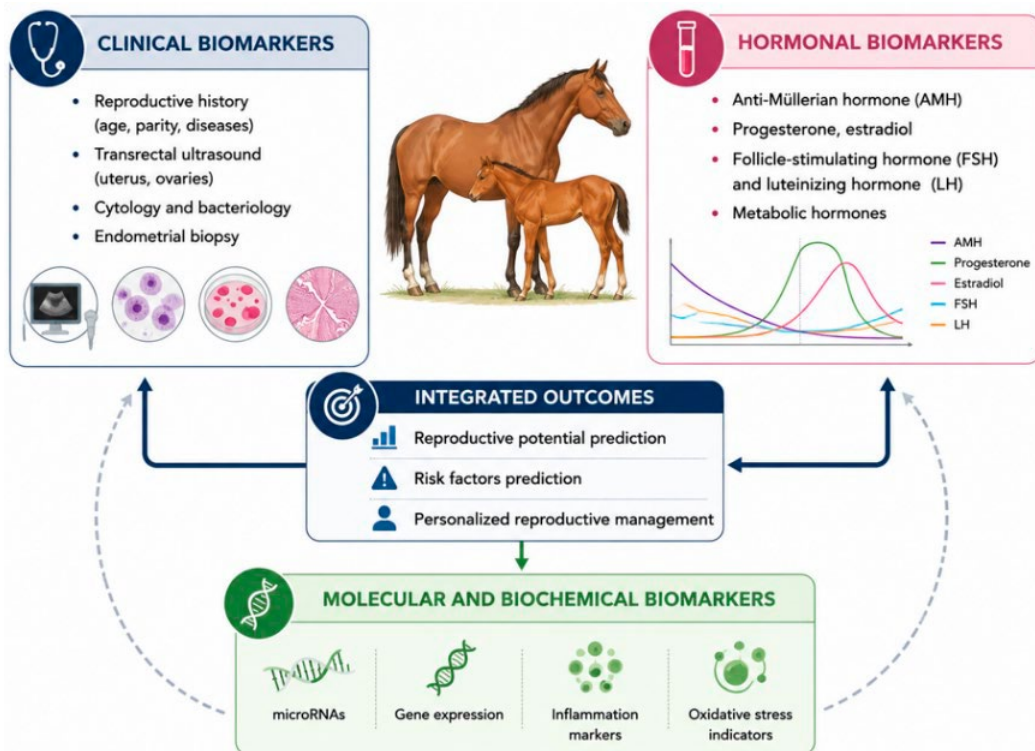


Fig. 5. Integrated biomarker-based assessment of reproductive potential in mares (adapted from Ball et al., 2008; Morris et al., 2020; Fouché et al., 2022; Fonte et al., 2024).

reserve and assisted reproduction performance (Ball et al., 2008; Canesin et al., 2023; Fonte et al., 2024). Nevertheless, interpretation of endocrine data requires consideration of cycle stage, season, age, and individual variability (Aurich, 2011; Cortés-Vidauri et al., 2018; Satué et al., 2021). Hormone concentrations represent dynamic physiological processes rather than static characteristics, which may partly explain the variability observed among studies evaluating their association with fertility outcomes (Goudet et al., 2022).

Recent advances in molecular biology have expanded understanding of reproductive processes beyond conventional clinical assessment. Cytokine profiles, oxidative stress indicators, extracellular matrix remodeling markers, transcriptomic signatures, and lipidomic profiles have all been linked to reproductive health and disease in mares (Fumuso et al., 2000; Ibrahim et al., 2021; Lawson et al., 2022; Silva-Álvarez et al., 2025). These approaches are particularly valuable because they may reveal biological alterations before overt clinical pathology becomes evident (Morris et al., 2020; Rebordão et al., 2021; Drzewiecka et al., 2025). At the same time, considerable methodological heterogeneity remains a major limitation. Differences in sampling procedures, analytical platforms, study populations, and outcome definitions complicate direct comparison of results and hinder the establishment of clinically meaningful reference values (Falomo et al., 2020; Lawson et al., 2022; Silva-Álvarez et al., 2025).

An important observation from the reviewed

literature is that biomarkers differ substantially in their level of validation and practical applicability. Clinical examination and selected hormonal markers are already integrated into routine reproductive management, whereas most molecular biomarkers remain primarily investigational (Katila, 2016; Ball et al., 2008; Fonte et al., 2024). This discrepancy does not diminish the biological importance of molecular findings; rather, it highlights the gap between mechanistic discovery and clinical implementation. Future studies should therefore focus not only on identifying new biomarkers but also on determining their reproducibility, diagnostic accuracy, and practical utility under field conditions (Fouché et al., 2022).

Integrating information from multiple biological levels may enhance reproductive decision-making, especially in mares with unexplained subfertility or inconsistent breeding outcomes (Papaas et al., 2021; Fouché et al., 2022). For example, assessing ovarian reserve using anti-Müllerian hormone may complement ultrasonographic evaluation (Ball et al., 2008; Fonte et al., 2024), while inflammatory and extracellular matrix biomarkers may provide additional insights into uterine health that are not apparent during routine examination (Schöniger and Schoon, 2020; Ferreira-Dias et al., 2024). Such combined approaches may enable more individualized breeding management and improve the selection of diagnostic or therapeutic interventions. However, evidence supporting specific biomarker combinations is limited, and prospective validation studies are needed before integrated biomarker panels can be

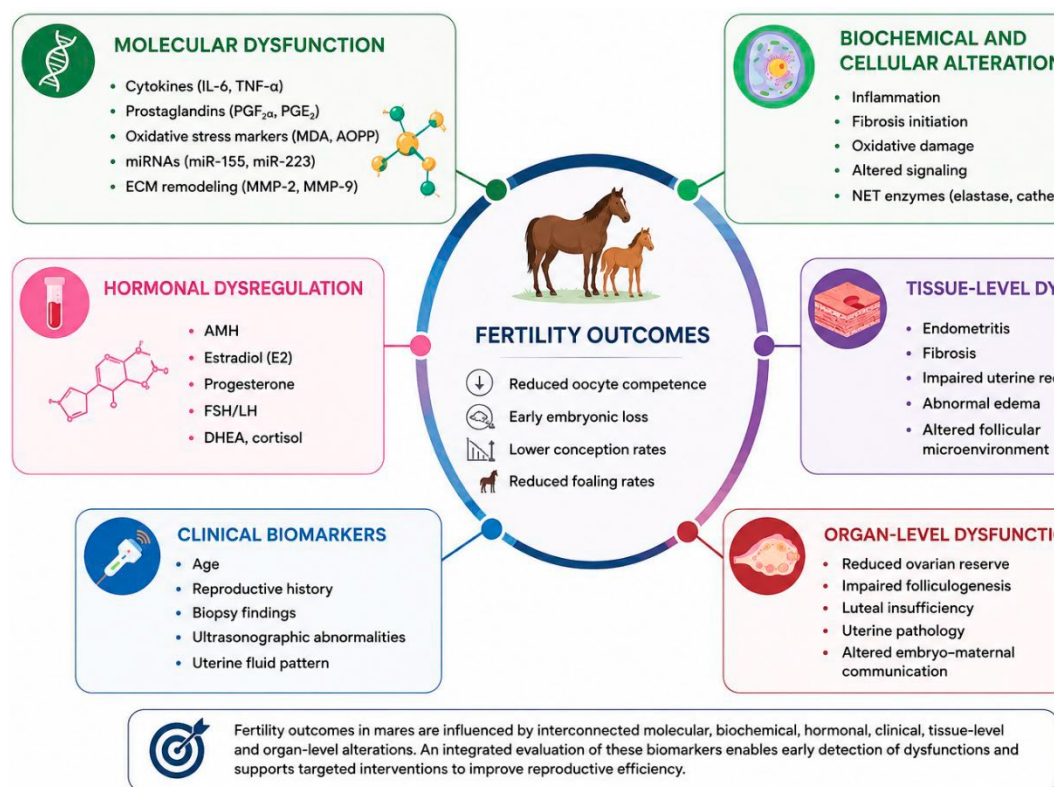


Fig. 6. Schematic representation of the biomarker cascade from molecular dysfunction to fertility outcomes in mares.

recommended for routine use (Fouché et al., 2022).

Future progress will require larger prospective studies, standardized sampling protocols, and greater methodological consistency across laboratories. Longitudinal investigations following mares through multiple breeding seasons may be particularly valuable, as they would allow evaluation of temporal biomarker dynamics and their relationship with reproductive outcomes (Ishak et al., 2019; Veronesi et al., 2025). Establishing robust reference ranges and validating promising molecular markers in clinically diverse populations should be considered essential priorities for future research (Lawson et al., 2022; Silva-Álvarez et al., 2025). The integration of clinical, hormonal, and molecular biomarkers and their collective contribution to reproductive potential assessment systems is illustrated in Fig. 5.

Fig. 5 summarizes the major biomarker categories currently investigated in mare reproductive assessment and illustrates their possible combined interpretation.

In Fig. 6, the central node is illustrated as the key fertility outcomes. Surrounding domains represent major contributors to reproductive dysfunction (Morris et al., 2020; Silva-Álvarez et al., 2025; Ferreira-Dias et al., 2024; Cecchini et al., 2023; Claes et al., 2017; Ishak et al., 2019; Rizzo et al., 2019; Hendriks et al., 2015; Canesin et al., 2023; Crabtree and Pycok., 2020; Katila, 2016; Ginther et al., 2008; Aurich et al., 2011; Fonte et al., 2024; Satué et al., 2021; Veronesi et al., 2025).

Conclusions

Mare reproductive potential is influenced by multiple interacting structural, endocrine, inflammatory, and cellular mechanisms. No single biomarker can adequately capture this complexity. Clinical examination remains the foundation of reproductive evaluation, while hormonal biomarkers provide additional information about ovarian reserve, follicular development, and luteal competence. Molecular and biochemical biomarkers may further enhance understanding of inflammatory activity, oxidative balance, tissue remodeling, and embryo-

maternal communication.

Available evidence indicates that combining clinical, endocrine, and molecular information offers a broader perspective on factors influencing fertility in mares; however, the predictive value of most integrated biomarker models has yet to be established. Most molecular biomarkers are insufficiently validated for routine clinical use and should therefore be interpreted with caution.

Future Perspectives

Future research should prioritize direct comparisons of biomarker categories under field conditions to determine whether combined biomarker panels improve fertility prediction beyond conventional reproductive examinations. It is especially important to conduct studies that directly compare the prognostic value of different biomarkers in real farm or field conditions. Further advancements in this area will depend on the use of standardized sampling protocols, larger prospective studies, and validation across various mare populations.

Multi-omics approaches may aid in the discovery of new biomarkers associated with ovarian aging, uterine receptivity, and early embryonic survival. However, for these methods to be routinely applied, they must be sufficiently reliable, accessible, and cost-effective for clinical use.

In the future, researchers working in this field should prioritize prospective longitudinal studies that assess biomarker dynamics across different breeding seasons in mares and directly link biomarker profiles to fertility outcomes. By identifying a standardized panel of metabolic markers in follicular fluid, researchers can go beyond mere correlation and identify causal factors that regulate oocyte developmental potential.

Emphasis should be placed on validating molecular biomarkers in larger, more clinically diverse cohorts of mares, establishing standardized reference ranges, and evaluating cost-effectiveness under field conditions. Such evidence will be required before biomarker-based decision tools can be incorporated into routine reproductive management programs.

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Abortion in Dromedary Camels (*Camelus dromedarius*) in Algeria: A Cross-Sectional Study

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Abstract. Abortions represent a major challenge for dromedary breeding in Algeria due to their economic, sanitary, and zoonotic implications. A cross-sectional study was conducted in El Oued province (southeastern Algeria) between 2022 and 2025 to investigate reproductive losses and associated risk factors in dromedary herds. The survey included 75 herds comprising 4460 females. Overall, 80% of the surveyed herds reported abortion cases. Among the affected herds, 8.3% (5/60) showed high abortion rates, 40% (24/60) showed frequent abortions, and 51.7% showed (31/60) sporadic cases. Stillbirths were also reported in 60% of these herds. The overall abortion rate was 8.52%, ranging from 7.8% to 10% in primiparous females and from 8.53% to 9.36% in multiparous females. Most abortions (90%) occurred during mid to late gestation, with a seasonal peak between August and November. Most breeders (88.3%) buried aborted fetuses and placentas, whereas 10% discarded them in the open environment and 1.7% used incineration. Following abortion events, 75% of breeders took no specific action, while only 25% sought veterinary assistance. Among the investigated risk factors, only the presence of domestic cats and dogs was significantly associated with abortion occurrence ($P < 0.05$). This study highlights a high frequency of abortions in Algerian dromedaries, accompanied by a notable proportion of stillbirths. To better elucidate the underlying etiological determinants as well as the associated risk factors, further studies combining epidemiological, clinical, pathological, and laboratory diagnostic approaches are required.

Introduction

In Algeria, the population of camels (*Camelus dromedarius*) plays a central role in livestock production systems (Faye, 2020). The dromedary is perfectly adapted to desert climates and extreme living conditions, thanks to its ability to efficiently utilize limited nutritional resources and convert them into edible products. It plays a key role in the national economy by providing essential products such as meat, milk and hides (Djeddou-Benabid et al., 2025; Djeddi et al., 2024). These animals play a crucial role in food security, providing a major source of animal protein, particularly for the populations of the arid regions of Algeria (Djeddou-Benabid et al., 2025).

Abortions constitute one of the most commonly observed problems in veterinary practice in camelids. Indeed, the birth rate generally does not exceed 40% in nomadic herds and 70% in intensive herds (Tibary et al., 2006). In addition to these low birth rates, camel herds experience significant neonatal losses, sometimes reaching epidemic proportions (Nawaz et Tharwat, 2025). The understanding of the incidence and etiology of abortions in camelids remains limited. Among the contributing factors, infectious, traumatic, and toxic agents appear to play a predominant role

(Tibary et al., 2006; Tibary et al., 2005).

Reproductive losses are mainly associated with abortion and early embryonic mortality, often linked to various infectious and parasitic agents involved in reproductive disorders. These losses may be exacerbated by unfavorable environmental conditions, particularly prolonged drought periods (Eggimann et al., 2024). Furthermore, dromedaries play an important role in the transmission of certain diseases to other livestock species as well as in the circulation of zoonotic diseases (Khan et al., 2003; Sazmand et al., 2019; Houssou et al., 2025). Abortion rates linked to infectious diseases range from 10% to over 70% in certain regions (Eggimann et al., 2024; Tibary et al. 2006).

Furthermore, abortion diagnosis remains challenging for both livestock producers and veterinarians, especially during the early stages when clinical signs are often absent or difficult to detect. Common indicators include the presence of placental tissues protruding from the vulva, the discovery of a dead fetus, or the unexpected return of estrus behavior (Fowler, 2011; Kandeel et Al-Mubarak, 2022).

To date, epidemiological data regarding reproductive losses in dromedary camels in Algeria, particularly abortions and stillbirths, remain very limited. The present study represents the first

large-scale retrospective investigation conducted in the Algerian Sahara to assess the extent of these reproductive disorders in camel herds.

The aim of this study was to conduct a comprehensive assessment of the magnitude of abortions and stillbirths in dromedary camel herds of the Algerian Sahara and to identify factors associated with the occurrence of these reproductive losses under local husbandry conditions. The specific objectives were to:

- estimate the prevalence of abortions and stillbirths at the herd level;
- describe their spatial and temporal distribution within the investigated herds;
- characterize the main zootechnical and reproductive parameters associated with these reproductive losses;
- identify potential risk factors involved in the occurrence of abortions;
- provide baseline data useful for improving health management and reproductive performance of dromedaries in the Algerian Saharan regions.

Materials and Methods

Ethical Approval

This study was based on a cross-sectional questionnaire and did not involve any experimental procedures, invasive sampling, or manipulation of animals. Therefore, ethical approval from an animal care and use committee was not required. Prior

informed consent was obtained from all herd owners before data collection.

Study Area

The study was conducted in El-Oued Province, located in southeastern Algeria (33°21'21" N, 6°51'47" E). Covering an area of 44 586.80 km², El-Oued is one of the largest administrative regions in the country, occupying a strategic position and serving as a major center for camel breeding and supply. It is bordered by Khenchela to the north, Tébessa to the northeast, Biskra to the northwest, Touggourt and M'ghair to the west, Ouargla to the south, and Tunisia to the east, with approximately 300 km of shared borders. The climate is Saharan according to the Köppen classification, characterized by very hot summers and mild winters. Annual temperatures range from an average minimum of 21.8°C to a maximum of 39°C, with a mean relative humidity of 24.3%. Rainfall is scarce, averaging between 80 and 100 mm per year and occurs mainly from November to February as moderate showers, occasionally accompanied by intense downpours (Fig. 1).

For the purpose of the analysis, the study area was stratified into two geographical zones based on administrative and spatial proximity criteria: a northern zone and a southern zone. The northern zone included the municipalities of Reguiba, Magrane Beni Guecha, Hassi Khalifa, Tayeb Larbi, Bayadah Nakhla, and El Oglia. The southern zone comprised

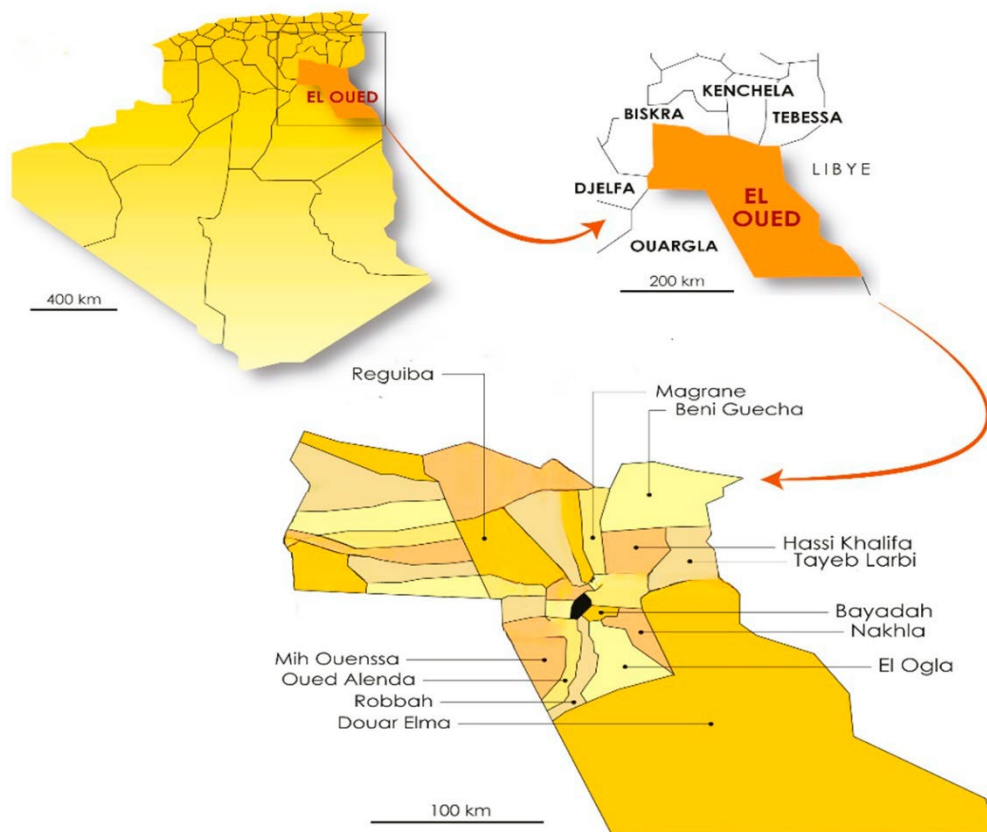


Fig. 1. Geographical distribution of the municipalities in El Oued province where the questionnaire survey was disseminated (Algeria Map)

Mih Ouensa, Oued Alenda, Robbah, Douar Elma, Bayadha, Nakhla, and El Oglia. This stratification was applied to assess potential geographical variation in abortion occurrence across the study area.

Sample Size and Herd Selection Procedure

The sample size was defined considering the number of dromedary camel herds available during the study period, with the aim of obtaining an adequate representation of abortion occurrence in the investigated region. Herds were selected from different geographical areas of the Algerian Sahara to ensure representative spatial coverage. A random selection procedure was applied based on the available herd lists, thereby ensuring an objective selection of the study units. The surveyed animals were adult female dromedaries of reproductive age (5 to 18 years), selected based on their available reproductive history and their presence within the herds included in the study.

A questionnaire was developed to collect information on reproductive events and potential factors associated with the occurrence of abortions in dromedary camel herds. An epidemiological survey was conducted among 75 camel breeders from 13 municipalities in El Oued Province. The questionnaire was administered through direct interviews with herd owners during field visits. The information provided by the respondents was recorded and subsequently used for epidemiological analysis.

Questionnaire Survey

An epidemiological cross-sectional survey was conducted in El Oued Province using a semi-structured questionnaire designed to collect standardized and detailed information. The questionnaire was structured into three main sections. The first section focused on herd characteristics, including herd size (female camels, male dromedaries, and young stock), husbandry system (extensive, intensive, semi-intensive), and herd replacement strategy (purchase, self-replacement, or mixed). The second section addressed reproductive performance, including herd-level abortion occurrence and stillbirths recorded during the periods 2022/2023 and 2024/2025. Information collected included the number of births and abortions per herd, parity status (primiparous and multiparous females), timing of abortion, gestational period (first, second, third and fourth gestational stages), type of expulsion (complete or incomplete), and management actions taken following abortion (none, isolation, or veterinary consultation). The third section recorded the presence of other domestic species (cattle, sheep, and goats), as well as the presence of cats and dogs within or around the herd environment.

In addition, open-ended questions were included to document clinical observations reported by breeders in aborted animals, the fate of the placenta and fetus,

and breeders' perceptions regarding possible causes of abortion. Herds were selected randomly within the study area.

Statistical Analysis

The questionnaire survey data were entered and coded using Microsoft Excel (Microsoft Corporation) and subsequently analyzed with SPSS software (version 27.0). A descriptive analysis was first performed to summarize the main characteristics of the studied variables.

Abortion frequency was calculated as the proportion of females that experienced abortion among the total number of pregnant females in each herd. Since no specific classification thresholds have been established for dromedary camels, abortion frequency categories were adapted from criteria previously applied in cattle (Givens and Marley, 2008) and classified as rare (< 2%), frequent (2–5%), or high (> 5%).

Herd-level abortion prevalence (%) was defined as the proportion of herds reporting at least one abortion case during the reproduction period, calculated by dividing the number of affected herds by the total number of herds surveyed and expressed as a percentage. Associations between herd-level abortion occurrence and potential risk factors were first assessed using Chi-square test or Fisher's exact test, as appropriate. In addition, univariable logistic regression analysis was performed to estimate odds ratios (OR) and 95% confidence intervals (CI) for each explanatory variable. A 95% confidence level was considered, and statistical significance was set at $P < 0.05$.

Results

Herd Characteristics and Reproductive Outcomes

The descriptive analysis included 75 camel breeders from 13 municipalities, representing a total population of 5870 dromedary camels, of which 4460 were females.

Herd size was distributed into six categories: [1–50], [50–100], [100–200], [200–300], [300–400], and [400–500], accounting for 44.0%, 33.3%, 16.0%, 1.3%, 4.0%, and 1.3%, respectively. Three husbandry systems were reported, namely extensive (62.7%), semi-intensive (28.0%), and intensive (9.3%). Herd replacement strategies were mainly self-replacement (58.7%), followed by mixed systems (29.3%) and purchase-based replacement (12.0%).

Among the surveyed breeders, 60 (80%) reported a history of abortion in their herds (Fig. 2). Among these affected herds, abortion frequency was classified as rare in 51.7% (31/60), frequent in 40.0% (24/60), and high in 8.3% (5/60).

Multiparous females showed higher abortion rates during the period 2022–2024, whereas primiparous females recorded a slightly higher rate in 2025.

Overall, abortion rates increased progressively throughout the study period, reaching their highest values in 2025 (Fig. 3).

At the individual female level, 502 abortions were recorded among 5886 pregnant females, corresponding to an overall abortion rate of 8.52% during the study period. The abortion rates according to year and parity group were as follows:

- 2022: primiparous (7.8%), multiparous (8.53%);
- 2023: primiparous (6.95%), multiparous (8.15%);
- 2024: primiparous (7.71%), multiparous (8.73%);
- 2025: primiparous (10%), multiparous (9.36%).

The spatial distribution of abortion cases across municipalities in El Oued Province is presented in Fig. 4.

Monthly abortion occurrence showed a progressive increase from July (16.7%) and August (25%), reaching a peak in September (53.3%), followed by

October (48.3%) and November (28.3%), before declining in December (8.3%), with lower values recorded earlier in the year. Regarding gestational stage, abortions were mainly observed in the third trimester (65%), followed by the fourth trimester (25%), while lower proportions were recorded in the second (6.7%) and first trimesters (3.3%). Placental expulsion was complete in 90% of cases. The placenta and aborted fetuses were mainly buried (88.3%), while 10% were discarded in open desert areas and 1.7% were incinerated. Following abortion events, 75% of breeders reported no intervention, whereas 25% sought veterinary consultation.

Clinical signs reported in aborted females included lethargy (15%), fever (11.7%), anorexia (10%), weight loss (6.7%), stress (5%), neurological signs (3.3%), agitation (3.3%), pulmonary inflammation (1.7%), and weakness (3.3%). However, 43.3% of breeders

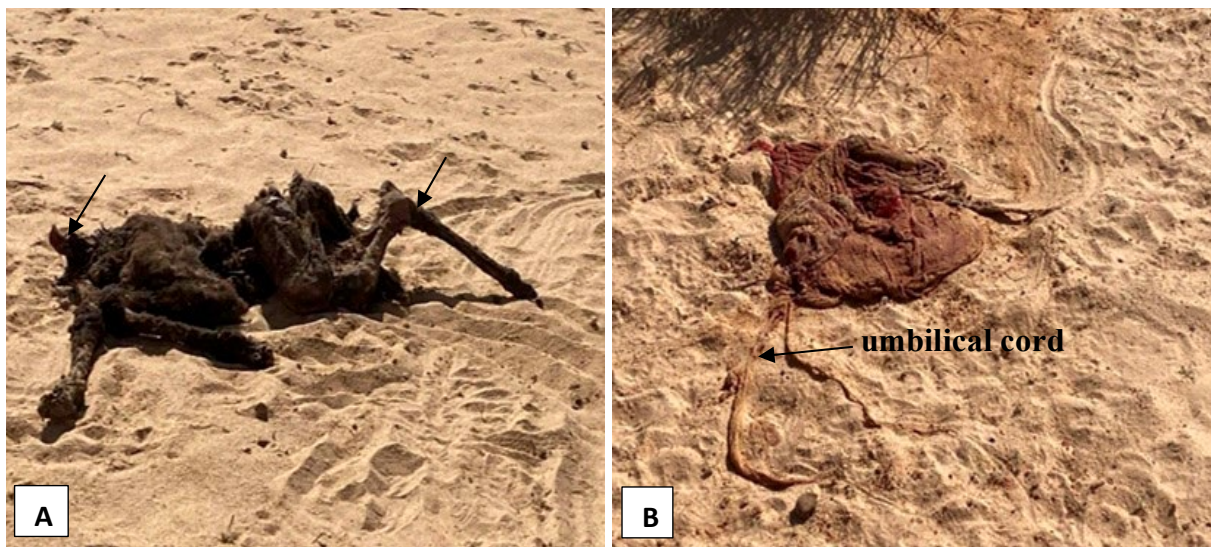


Fig. 2. A case of late-term abortion (11 months) observed during our survey in the Saharan region of El Oued province.

A – Aborted fetus in an advanced state of desiccation with absence of the head and exposed bones of the hind limbs (arrows), suggesting scavenging by wild animals. B – Placenta from the she-camel that aborted.

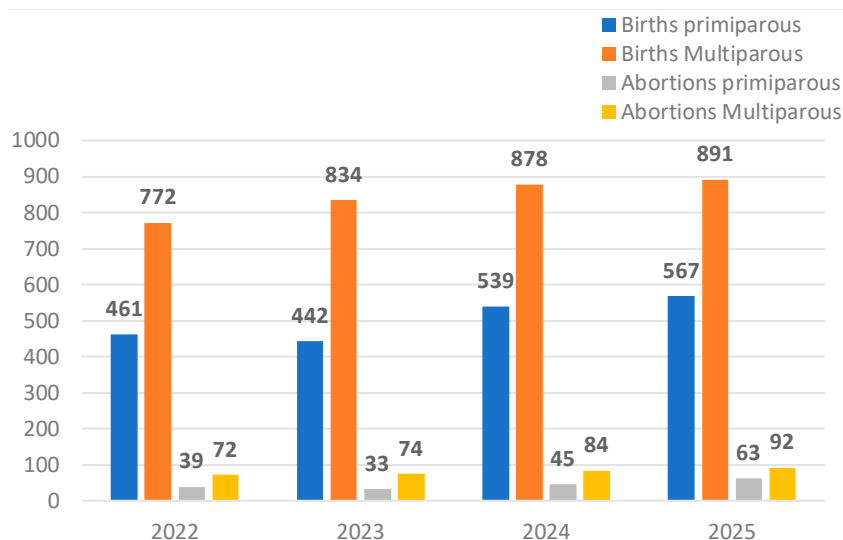


Fig. 3. Evolution of births and abortions among female dromedary camels in El Oued

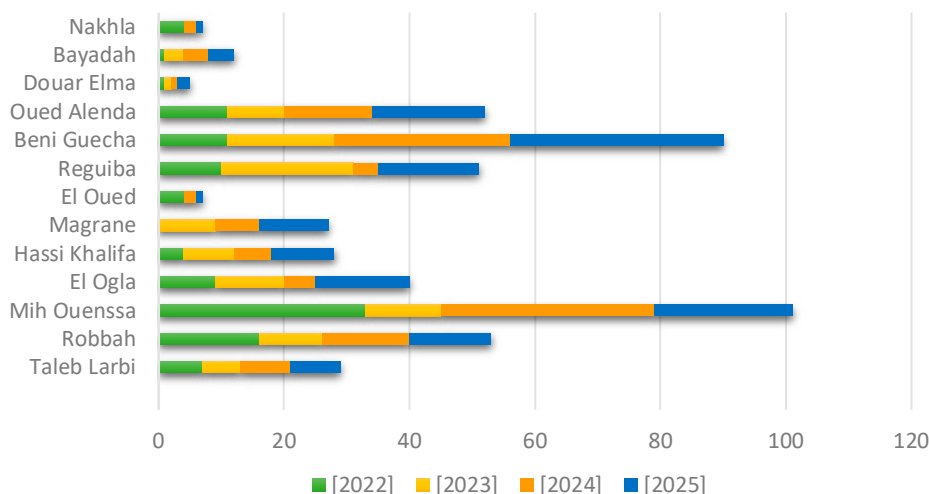


Fig. 4. Incidence of abortions in female dromedary camels by municipality in El Oued

reported no observable clinical signs. Breeders reported multiple perceived causes of abortion, including drought (55%), insect infestation (28.3%), consumption of contaminated water (23.3%), various diseases (23.3%), malnutrition (16.7%), excessive heat (13.3%), fever (6.7%), poor management practices (3.3%), anorexia (1.7%), and plant toxicity (1.7%).

Among the 60 abortion-affected herds, the presence of other animal species was reported, including cattle (5%), sheep (58.3%), goats (60%), dogs (71.6%), and cats (80%). In addition, 13 breeders reported possible contact with stray dogs and cats. Among the 60 breeders reporting abortion cases, 36 (60%) also reported stillbirths during the 2022–2025 period, while 3 (5%) observed neonatal mortality within 2–6 days after birth, and 21 (35%) reported no such events. In cases of stillbirth, reported observations included fetal swelling (6%), fever (6%), stress in the last three days before parturition (3%), and anemia/cachexia (3%). However, 81.8% of breeders reported no associated clinical signs.

Association Analysis of Risk Factors

Most of the investigated factors showed no statistically significant association with abortion in camel herds ($P > 0.05$), including geographic zone, variables such as the presence of cattle, sheep, and goats, as well as management-related factors including husbandry system and herd replacement strategy. These results indicate weak statistical evidence supporting an association between abortion occurrence and the majority of structural or management characteristics assessed.

In contrast, A strong and statistically significant association was observed between the presence of cats and herd-level abortion occurrence (OR = 11, 95% CI: 2.98–40.67; $p < 0.001$), indicating that herds with cats had approximately 11-fold higher odds of abortion occurrence compared with herds without cats. Similarly, the presence of dogs was significantly associated with abortion occurrence (OR = 3.79, 95%

CI: 1.17–12.30; $p = 0.033$), with herds where dogs were present showing approximately 3.8-fold higher odds of abortion compared with herds without dogs (Table 1, Fig. 5).

Discussion

General and Reproductive Characteristics of the Herds

The camel herds investigated in this study were mainly small-sized herds, with a predominance of breeding females. This structure reflects the typical management strategy of camel production systems, in which maintaining a high proportion of females is essential to ensure reproduction and herd sustainability. Similar patterns have been reported by Megersa et al. (2008) and Al-Dahash and Sassi (2009). The predominance of extensive management and reliance on self-replacement indicate a low-input production system mainly based on natural herd renewal, which may contribute to maintaining herd stability. However, these systems may also limit reproductive monitoring and health control measures, potentially affecting reproductive performance, particularly abortion occurrence, as reported in previous studies (Adamou, 2008; Mayouf et al., 2014).

Data on abortions in dromedaries in North Africa remain limited. To the best of our knowledge, this study represents one of the first epidemiological surveys conducted in Algeria in this field, providing baseline data that may serve as a reference for future research in this context.

A high proportion of breeders reported abortion cases in their herds, while the use of veterinary services remained limited. This finding, also reported by Khalafalla et al. (2017), suggests that reproductive losses may be insufficiently diagnosed and managed in camel herds. In dromedaries, where reproductive performance is naturally limited by delayed sexual maturity, seasonal breeding activity, and prolonged gestation, abortions may have a significant impact on herd productivity by extending the calving interval.

Table 1. Analysis of potential risk factors associated with abortion in female dromedaries

Variables	Categories	Total Herds	No of Unaffected Herds	No of Affected Herds	OR (95% CI)	P value
Geographic zone	North	34	5	29	1 (ref)	0.56
	South	41	10	31	0.53 (0.16–1.75)	
The presence of cattle	No	68	11	57	1 (ref)	0.13
	Yes	7	3	4	0.26 (0.05–1.31)	
The presence of sheep	No	35	10	25	1 (ref)	0.093
	Yes	40	5	35	2.80 (0.85–9.20)	
The presence of goats	No	31	7	24	1 (ref)	0.771
	Yes	44	8	36	1.31 (0.42–4.10)	
The presence of dogs	No	26	9	17	1 (ref)	0.033*
	Yes	49	6	43	3.79 (1.17–12.30)	
The presence of cats	No	23	11	12	1 (ref)	< 0.001*
	Yes	52	4	48	11 (2.98–40.67)	
Husbandry system	Extensive	45	10	35	1 (ref)	0.075
	Intensive	7	4	3	0.21 (0.04–1.12)	
	Semi-intensive	23	1	22	6.29 (0.75–52.56)	
Herd replacement strategy	Purchase	9	3	6	1 (ref)	0.37
	Self-replacement	44	8	36	2.25 (0.46–10.96)	
	Mixed	22	4	18	2.25 (0.39–13.07)	

No, number; OR, odds ratio; CI, confidence interval; *, significant,

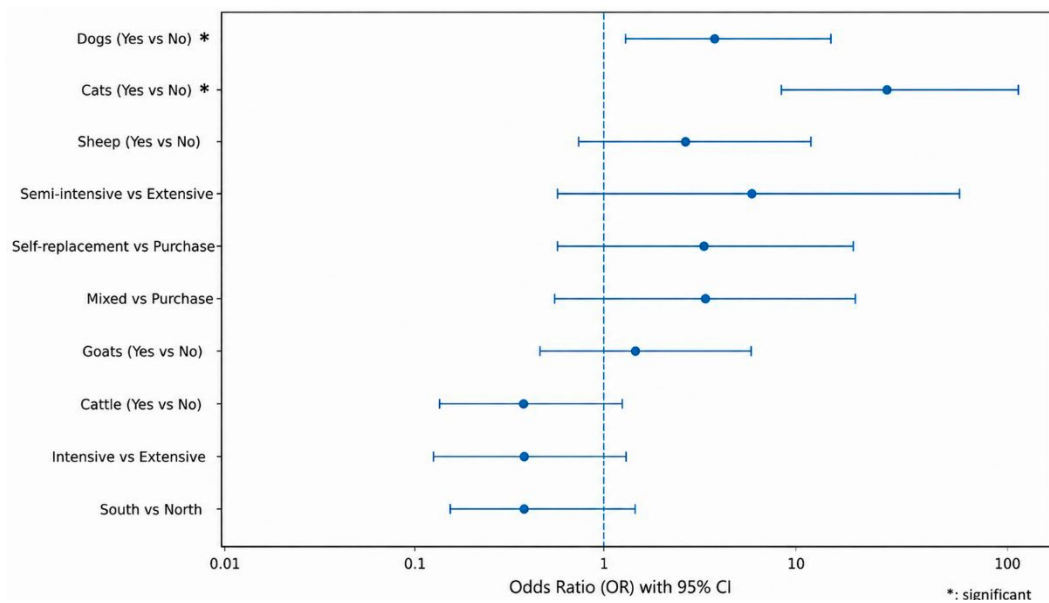


Fig. 5 .A forest plot of risk factors associated with herd-level abortion

These findings highlight the importance of improving reproductive monitoring and strengthening access to veterinary services in camel production systems (Monaco et al., 2015; Faye et al., 2022).

The limited use of veterinary services in cases of abortion likely reflects gaps in animal health awareness, insufficient veterinary outreach, and limited integration of camel production systems into

formal veterinary surveillance programs. In addition, assessment of carcass and tissue disposal practices showed that only a small proportion of breeders failed to properly dispose of aborted materials, suggesting a relatively moderate level of awareness regarding potential health risks.

Multiparous females recorded higher numbers of both births and abortions compared with primiparous

females, which is expected given their reproductive history. However, the persistently higher abortion frequency in multiparous females may also reflect cumulative exposure to infectious agents or age-related physiological changes. A gradual increase in abortion cases was observed in 2025, particularly among primiparous females, which may indicate increased susceptibility potentially associated with environmental stressors or underlying reproductive disorders.

During the study period, the overall abortion rate observed was higher than those reported in previous studies by Al-Dahash and Sassi (2009) and Nagy et al. (2023) but remained lower than the levels described in Niger by Gilles et al. (2006) and in Chad by Touka et al. (2025). These discrepancies between studies may be attributed to several factors, including differences in sampling strategies, herd management systems, production conditions, climatic parameters, as well as variations in the prevalence of infectious and non-infectious reproductive disorders.

Overall, most farms showed relatively low abortion frequencies, suggesting that abortion is not uniformly distributed across herds but rather concentrated in specific vulnerable populations. However, early embryonic losses are likely underreported due to difficulties in detection, which may lead to the underestimation of true reproductive losses. Previous studies have reported that early pregnancy losses may be substantial in camels (Toolan, 2003; Henker et al., 2022). Tibary et al. (2006) further reported a wide variability in reproductive performance, with pregnancy or calving rates ranging from 12% to 85% and abortion rates reaching up to 40%.

Abortion occurrence showed a seasonal distribution, with a noticeable increase during September and October. This variation may be associated with the combined influence of several factors, including climatic conditions, feed availability, nutritional status of females, herd management practices, and seasonal dynamics of infectious agents. Similar trends were reported by Nagy et al. (2023), whereas other studies described peaks occurring at different periods, including June or early spring (Gilles et al., 2006). These differences suggest that the seasonality of abortion in dromedaries depends on geographical context, production systems, and local epidemiological conditions, confirming the multifactorial nature of this phenomenon.

Incomplete expulsion was observed in a small proportion of cases, in agreement with the findings reported by Al-Dahash and Sassi (2009). Although uncommon, this complication may contribute to postpartum disorders and highlights the importance of appropriate reproductive management following abortion events.

Most abortions occurred during the advanced stages of gestation, suggesting a greater involvement of factors affecting the maintenance of late pregnancy.

Similar observations were reported by Nagy et al. (2023). This pattern may be associated with several factors, including infections, nutritional conditions, maternal stress, or placental disorders, whose impact may vary according to herd management practices and the local epidemiological context.

Finally, more than half of the breeders reporting abortion also reported stillbirths within their herds. This concurrent occurrence may reflect shared underlying risk factors affecting gestation and perinatal survival, potentially involving infectious, environmental, or management-related determinants.

Risk Factors Associated with Abortion in Camel Herds

The absence of significant associations between abortion occurrence and most of the investigated variables, including geographic zone, herd composition, husbandry system, and herd replacement strategy, indicates that these factors were not statistically related to abortion under the conditions of the present study. In contrast, the presence of dogs and cats showed significant associations at the herd level. However, given the questionnaire-based nature of the study and the absence of clinical, pathological, and laboratory investigations, these findings should be considered as potential epidemiological indicators. Overall, the results suggest that additional factors not assessed in the present study, including environmental, infectious, or herd health management-related factors, may also contribute to the observed variability in abortion occurrence.

Potential Causes of Abortion in Dromedary Camels

The diversity of observations reported by breeders indicates that abortions in dromedaries may occur under various conditions. Clinical manifestations such as fever, weight loss, and other non-specific signs were reported in some cases, whereas a substantial proportion of breeders reported no apparent signs associated with abortion events. These observations may reflect the heterogeneous nature of abortion occurrence in camel herds; however, the absence of clinical examinations and diagnostic confirmation does not allow the underlying causes to be determined.

Several factors potentially responsible for abortions were reported in this survey, including drought, the presence of insects, diseases, wastewater contamination, and feed shortages. The relative importance of these factors varied considerably across municipalities, highlighting the key role of environmental conditions and local management practices in abortion risk. Indeed, the prolonged drought observed in the study area has led to significant food shortages, notably a marked decline in forage vegetation. This situation has harmful effects on the animals, resulting in weight loss and weakened immunity (Adamou, 2008), which

may compromise the reproductive performance of she-camels and lead to abortions and stillbirths. Other non-infectious factors were also reported by Nagy et al. (2023), including fetal and placental abnormalities (such as twinning, umbilical cord torsion, placental insufficiency and genetic defects), endocrine disorders, and various environmental factors, such as nutritional deficiencies, toxicosis, trauma and maternal diseases.

Infectious agents play a major role in the occurrence of abortions, with incidence rates showing significant regional disparities, ranging from 10% to over 70% depending on the geographic area. According to Tibary et al. (2006), brucellosis and trypanosomiasis (mainly transmitted through insect bites) are the main causes of infectious abortion in camels, particularly in the Middle East and Africa. In contrast, in llamas and alpacas, abortions are mainly attributed to leptospirosis, toxoplasmosis and chlamydiosis. The distribution of both infectious and non-infectious causes of abortion varies across countries and breeding seasons. Nevertheless, a considerable proportion of cases (8% to 35%) remain of unknown etiology (Nagy et al., 2021). Overall, the reviewed studies consistently show that reproductive losses in dromedaries are associated with a wide range of infectious agents, with marked geographical variability in their relative importance. In Algeria, serological data have highlighted exposure to several abortive pathogens, including *Brucella* spp., *Trypanosoma evansi*, *Chlamydia abortus* and BoHV-1 (Benaissa, 2015). Similarly, in Egypt and Saudi Arabia, multiple bacterial, viral and protozoan agents have been identified as causes of abortion, stillbirth and neonatal mortality, including *Toxoplasma gondii*, *Theileria* spp., *Anaplasma* spp., *Chlamydia psittaci*, *Coxiella burnetii*, *Campylobacter* spp., *Salmonella* spp., as well as bovine viral diarrhea virus (Osman et al., 2016; Khalafalla et al., 2017; Khalifa et al., 2018). In addition, studies from Iran confirm that the main causes of fetal losses include brucellosis, trypanosomiasis, leptospirosis,

salmonellosis, BVD, IBR and aspergillosis (Hassani, 2021). Furthermore, protozoal infections such as toxoplasmosis, neosporosis, sarcocystosis and trypanosomiasis are also recognized as important zoonotic causes of abortion in camels (Shaapan, 2016). Taken together, these findings highlight that abortions in camels are of multifactorial origin, involving a complex interaction between bacterial, viral, protozoal and possibly environmental factors, which makes their diagnosis and control particularly challenging.

This study presents several limitations that should be considered when interpreting the findings. Its cross-sectional questionnaire-based design does not allow causal inference and limits the interpretation of the results to statistical associations. In addition, data based on breeders' reports may be subject to recall bias and potential misclassification of reproductive events and exposure variables. Furthermore, the absence of clinical, pathological, and laboratory investigations did not allow confirmation of the etiological causes of abortion. Consequently, the present findings should be interpreted strictly at an epidemiological level, and further studies integrating epidemiological, clinical, pathological, and laboratory approaches are required to better characterize the factors involved in abortion in dromedaries.

Conclusion

This study highlights a high frequency of abortions in dromedary camel herds, accompanied by a notable proportion of stillbirths. Most factors related to herd characteristics and management practices showed no significant association with abortion occurrence within the framework of this survey. These findings should be interpreted with caution due to the observational and declarative nature of the data. In this context, further investigations integrating epidemiological, clinical, pathological, and laboratory diagnostic approaches are required to better understand the underlying mechanisms of abortion in dromedaries and to guide appropriate preventive strategies.

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The Effects of Vitamins C and E against Chlorpyrifos Toxicity on *Capra hircus* Spermatozoa: Involvement of Oxidative Stress

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Keywords: chlorpyrifos, *Capra spermatozoa*, oxidative stress, vitamin C, vitamin E.

Abstract. Chlorpyrifos (CPF), a widely used broad-spectrum pesticide, has been shown to affect significantly fertility outputs. However, there is limited knowledge concerning the potential involvement of oxidative stress (OS) in CPF-induced sperm dysfunction in mature spermatozoa and the alternatives to counteract the potential deleterious effects. This study aimed, on the one hand, to investigate the effects of CPF on sperm motility and to assess whether OS may be involved in CPF-induced toxicity in epididymal spermatozoa. On the other hand, it aimed to evaluate the effects of vitamin C and vitamin E, administered individually or in association in the presence of CPF. Epididymal sperm was collected from five *Capra hircus* testes and divided into five equal-volume aliquots, receiving different treatments: Control–, CPF, VitC+CPF, VitE+CPF and VitCE+CPF. Control– group was diluted in TRIS extender, while CPF group was exposed to CPF (50 µg/mL). The vitamin-treated groups were pre-incubated for 20 min with vitamin C (0.1 mg/mL), vitamin E (0.25 mg/mL), or their combination prior to CPF exposure. Sperm motility was assessed after 0, 30, and 60 min of storage at 37°C using computer aided sperm analysis. Malondialdehyde (MDA) levels were measured at 30 min in each treatment by the thiobarbituric acid reactive substances (TBARS) assay and quantified at 535 nm. CPF exposure significantly impaired sperm kinetics; at 60 min, VSL decreased from 11.248 ± 0.209 µm/s in the Control– group to 5.051 ± 0.143 µm/s in the CPF group ($P < 0.0001$). Also, progressive motility decreased in the CPF group ($0.225 \pm 0.084\%$) compared with the Control– group ($4.146 \pm 1.224\%$). VitCE+CPF improved significantly VSL compared with CPF group (17.405 ± 0.185 µm/s vs CPF; $P < 0.0001$). MDA levels were elevated significantly in the CPF group (2.261 ± 0.108 nmol MDA/ 10^8 SPZ) compared with the Control– group (1.919 ± 0.109 nmol MDA/ 10^8 SPZ; $P = 0.035$). VitCE+CPF resulted in the lowest MDA concentration (1.739 ± 0.107 nmol MDA/ 10^8 SPZ vs CPF; $P = 0.003$). The results showed that CPF affected mature spermatozoa, accompanied by an increase in lipid peroxidation. These effects may be attenuated in the presence of vitamins C and E, especially when they are used in association.

Introduction

Pesticides find extensive application in agriculture and industry owing to their capacity to protect crops during growth from insects, weeds, fungi, and bacteria (Gangola et al., 2022; Sule et al., 2022). However, they are considered one of the most important contaminants worldwide due to their persistence and toxicity, and numerous studies have reported their detrimental effects on the environment and human health (Gangola et al., 2022; González-Curbelo et al., 2022). Chlorpyrifos (O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothionate) (CPF), an organophosphate pesticide, is one of the most widely used broad-spectrum pesticides and is classified as a moderately hazardous class II insecticide (Alaa-Eldin et al., 2017). It is mainly used in agriculture to control both foliage, soil-borne pests affecting crops, flea

treatments, and in residential settings (Sabarwal et al., 2018; Singh et al., 2018). Many epidemiological studies have reported a correlation between exposure to organophosphorus (OP) molecules and reproductive disorders (Dhanushka and Peiris, 2017; Chhillar et al., 2023). In vivo, Alaa-Eldin et al. (2017) found that chlorpyrifos and cypermethrin induced reproductive toxicity in albino rats affecting testicular weight, sperm motility, viability, and count. Similarly, Joshi et al. (2007) reported that subchronic exposure to CPF at doses of 7.5, 12.5, and 17.5 mg/kg/day for 30 days altered male rats' fertility by causing severe testicular damage, declining sperm count and motility and decreasing serum testosterone levels. These findings were further supported by Farag et al. (2010), who reported similar results in male mice treated with CPF at 15 or 25 mg/kg/day for 4 weeks.

It has been established that OP pesticides exert their toxicity mainly by inhibiting acetylcholinesterase,

resulting in the accumulation of acetylcholine at the cholinergic nerve terminal (Dhanushka and Peiris, 2017; Lushchak et al., 2018). Other putative mechanisms, including oxidative stress (OS), have also been reported. In particular, chlorpyrifos may induce OS by generating free radicals, altering antioxidant defense (Uchendu et al., 2018), and consequently inducing reproductive toxicity (Mandal and Das, 2011). In fact, it has been highlighted that OS is considered a potential cause of male infertility (Latchoumycandane et al., 2002; Altaher et al., 2023), causing substantial damage during spermatogenesis and leading to lipid, protein, and DNA alterations (Moridi et al., 2018; Altaher et al., 2023). Recently, Talebinasab et al. (2025) have found that male rats exposed to CPF exhibited testicular toxicity with increased MDA levels and decreased activity of antioxidant enzymes.

Numerous researchers have investigated various approaches to overcome such adverse effects on reproductive function. In particular, antioxidant supplementation has been highlighted as a promising approach to mitigate the damaging effects of pesticides. Antioxidants serve as agents that eliminate, scavenge, and inhibit the generation of ROS (Sheweita et al., 2005). However, as the endogenous antioxidant system may not be sufficient to protect cells from OS, antioxidant supplementation is widely considered an effective alternative (Sule et al., 2022). Thus, vitamin E, also known as α -tocopherol, and vitamin C have emerged as effective scavengers of ROS (Mukai et al., 1993; Bhardwaj et al., 2018). In this regard, Aly et al. (2010) suggested that pre- or post-administration of vitamin C in male mice exposed to CPF prevented oxidative damage. Jaiswal et al. (2013) also showed that vitamin C supplementation significantly reduced OS markers in the hearts of rats exposed to carbofuran. However, the association of vitamins C and E appears to provide better protection against OS damage in different experimental reports. In this respect, vitamins C and E have been shown to improve pregnancy rates in couples (Zhou et al., 2022) and to protect spermatogenesis against endosulfan (Takhshid et al., 2012).

However, to the best of our knowledge, no previous studies have investigated either the direct impact of chlorpyrifos (CPF) on mature spermatozoa using an in vitro model or the potential effects of vitamins C and E on CPF-induced sperm alterations. The present study aimed first to assess CPF's effects on sperm parameters without considering other associated factors, including hormonal secretion or testicular integrity, which may be involved in vivo. Second, the study aimed to investigate whether oxidative stress may contribute to CPF-induced toxicity, as assessed by lipid peroxidation, and to examine the effects of vitamins C and E, administered individually or in association.

Materials and Methods

Ethical Statement

Testes from adult *Capra hircus* were obtained from a local slaughterhouse following routine slaughter. The study involved only in vitro experiments on semen samples and did not require direct animal experimentation. All procedures were conducted in accordance with institutional guidelines and good veterinary practice.

Semen Collection and Preparation

Testes were collected from five adult goats (*Capra hircus*, two years old) obtained from a local slaughterhouse in Béjaïa (Algeria). One testis from each animal was used in the present study (N = 5). Immediately after slaughter, testes were collected and transported to the laboratory in a sterile airtight container maintained at approximately 37°C. The experimental procedures were initiated within 1 h post-slaughter. Semen was obtained by the retrograde flushing method as previously described by Martinez-Pastor et al. (2006). The epididymis was meticulously isolated from the testis and rinsed using a TRIS extender. An incision was made at the cauda of the epididymis, and measured pressure was applied through the introduction of 1 mL of TRIS extender into the lumen of the vas deferens.

Chemicals

All chemicals, including Tris(hydroxymethyl)aminomethane, fructose, citric acid, polyethylene glycol 6000, trichloroacetic acid (TCA), and thiobarbituric acid (TBA), were obtained from Sigma-Aldrich (St. Louis, MO, USA). Vitamin C (ascorbic acid) and vitamin E (α -tocopherol) were also purchased from Sigma-Aldrich (St. Louis, MO, USA). The chlorpyrifos used in this study was PYRICAL 480 EC, which is a concentrated emulsifiable formulation containing 480 g/L of chlorpyrifos, supplied by ARYSTA Life Science.

In Vitro Exposure of Spermatozoa to Chlorpyrifos/Pre-Incubation with Vitamins

In this study, a TRIS extender composed of 1.510 g Tris(hydroxymethyl)aminomethane, 0.625 g fructose, 0.850 g citric acid, 0.050 g penicillin G dissolved in 50 mL of distilled water was used for semen dilution.

Each semen sample was divided into five equal-volume aliquots (10 μ L) to evaluate the effects of five experimental treatments:

- Control– served as the negative control and contained only TRIS extender.
- CPF served as the positive control, receiving CPF exposure without antioxidant supplementation.
- VitC+CPF was supplemented with vitamin C (0.1 mg/mL; 0.57 mM) 20 min before exposure to CPF.
- VitE+CPF was supplemented with vitamin E

(0.25 mg/mL; 0.58 mM) 20 min before exposure to CPF.

- VitCE+CPF was supplemented with a combination of both vitamins, vitamin C (0.1 mg/mL; 0.57 mM) and vitamin E (0.25 mg/mL; 0.58 mM), 20 min before exposure to CPF.

The experimental design consisted of two sequential dilution steps. In the first step (1:100), 10 μ L of fresh semen was added to 990 μ L of treatment medium (TRIS extender or vitamins), and the mixture was incubated at 37°C for 20 min. A second dilution step (1:2) was then performed by adding CPF (50 μ g/mL) (Pallotta et al., 2018) to corresponding groups, except the Control–.

To enhance its solubility, vitamin E was solubilized in polyethylene glycol 6000 (PEG 6000) at a 10:90 (w/w) ratio, as described by Amokrane et al. (2020).

Computer-assisted Sperm Analysis (CASA)

Sperm analysis was performed in each sample at 0, 30, and 60 min using the Sperm Class Analyzer[®] (SCA) Version 5.4 (Microptic S.L. Viladomat 321, 6e408029, Barcelona, Spain). For each sample, 10 μ L of sperm was analyzed using an analysis chamber with a depth of 10 μ m (Makler Counting Chamber, Sefi-Medical Instruments Ltd., Biosigma S.r.l., Italy) maintained at 37°C. Acquisition was performed at 25 frames per second with a 10 \times objective using a phase contrast microscope. The standard CASA settings included a head area range of 3–70 μ m². Spermatozoa were considered motile when curvilinear velocity (VCL) exceeded 10 μ m/s. The sperm kinetic parameters evaluated were curvilinear velocity (VCL, μ m/s), average path velocity (VAP, μ m/s), and straight-line velocity (VSL, μ m/s). Sperm motility parameters included progressive motility (PM, %) and rapid motility (R, %).

Measurement of Malondialdehyde

The malondialdehyde (MDA) level was assessed in each treatment as an indicator of lipid peroxidation using the thiobarbituric acid (TBA) assay as described by Buege and Aust (1978), adapted by introducing sperm washing, standardization of sperm concentration (100×10^6 spermatozoa/mL), as well as reagent volumes and centrifugation conditions. Absorbance was measured using a Jenway Genova spectrophotometer (Jenway Ltd., Essex, UK). Prior to TBARS analysis, sperm concentration in each sample was determined by CASA and adjusted to 100×10^6 spermatozoa/mL to ensure comparability among treatments.

Briefly, after 30 min of co-incubation, all samples were centrifuged at $800 \times g$ for 10 min to separate the spermatozoa from the treatment media. Distilled water was added to the pellets, and the samples were centrifuged at $6000 \times g$ for 15 min. Then, 315 μ L of trichloroacetic acid (TCA) and 125 μ L of TRIS extender were added to each supernatant. All samples

were incubated at 0°C for 2 h, then centrifuged at $16\,000 \times g$ for 10 min. Subsequently, 150 μ L of TBA was added to the supernatant of each sample.

The samples were boiled for 15 min and then cooled in an ice bath. Finally, absorbance was measured at 535 nm. The molar extinction coefficient for MDA was $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$. The results were expressed as nmol MDA/ 10^8 SPZ.

Statistical Analysis

Data were collected and expressed as mean \pm SEM. Normality of data distribution was assessed prior to statistical analysis for all parameters. Kinetic parameters (VCL, VAP, VSL) did not satisfy the normality assumption ($P < 0.05$) and were therefore analyzed using the Kruskal–Wallis test followed by the Mann–Whitney U test with Bonferroni corrections performed in Stat View (SAS Institute Inc.). Progressive (PM), rapid (R) motility, and MDA levels satisfied the normality assumption ($P > 0.05$) and were therefore analyzed using ANOVA (GraphPad Prism, version 8.0.2). Two-way ANOVA followed by Dunnett's multiple comparisons test was applied to PM and R, assessed at three time points (0, 30, and 60 min), with the CPF group as reference. One-way ANOVA with Dunnett's multiple comparisons test was applied to MDA levels, measured at a single time point (30 min), with the CPF group as reference. Differences were considered statistically significant at $P < 0.05$.

Results

Kinetic Parameters of Spermatozoa Co-Incubated with Chlorpyrifos and Vitamins

CPF exposure significantly reduced VCL, VAP, and VSL compared with the Control– group ($P < 0.0001$), whereas treatment with vitamin C, vitamin E, or their association significantly increased these parameters compared with the CPF group ($P < 0.0001$) as shown in Fig. 1.

Among the vitamin-treated groups, the combined group VitCE+CPF showed the highest VCL, VAP, and VSL values across the 0, 30, and 60 min assessments.

At 60 min, the VitCE+CPF group showed VCL, VAP, and VSL values of 70.110 ± 0.522 , 31.563 ± 0.231 , and 17.405 ± 0.185 μ m/s, respectively. In contrast, the CPF group showed corresponding values of 30.347 ± 0.507 , 15.095 ± 0.247 , and 5.051 ± 0.143 μ m/s, respectively. Both VitC+CPF and VitE+CPF significantly improved sperm kinetic parameters compared with the CPF group ($P < 0.0001$). Mean values are summarized in Table 1.

Effects on the Percentages of Progressive and Rapid Motility

Fig. 2 illustrates the percentages of progressive (PM) and rapid (R) motility in the five experimental groups at 0, 30, and 60 min. CPF exposure significantly decreased rapid motility compared with the Control–

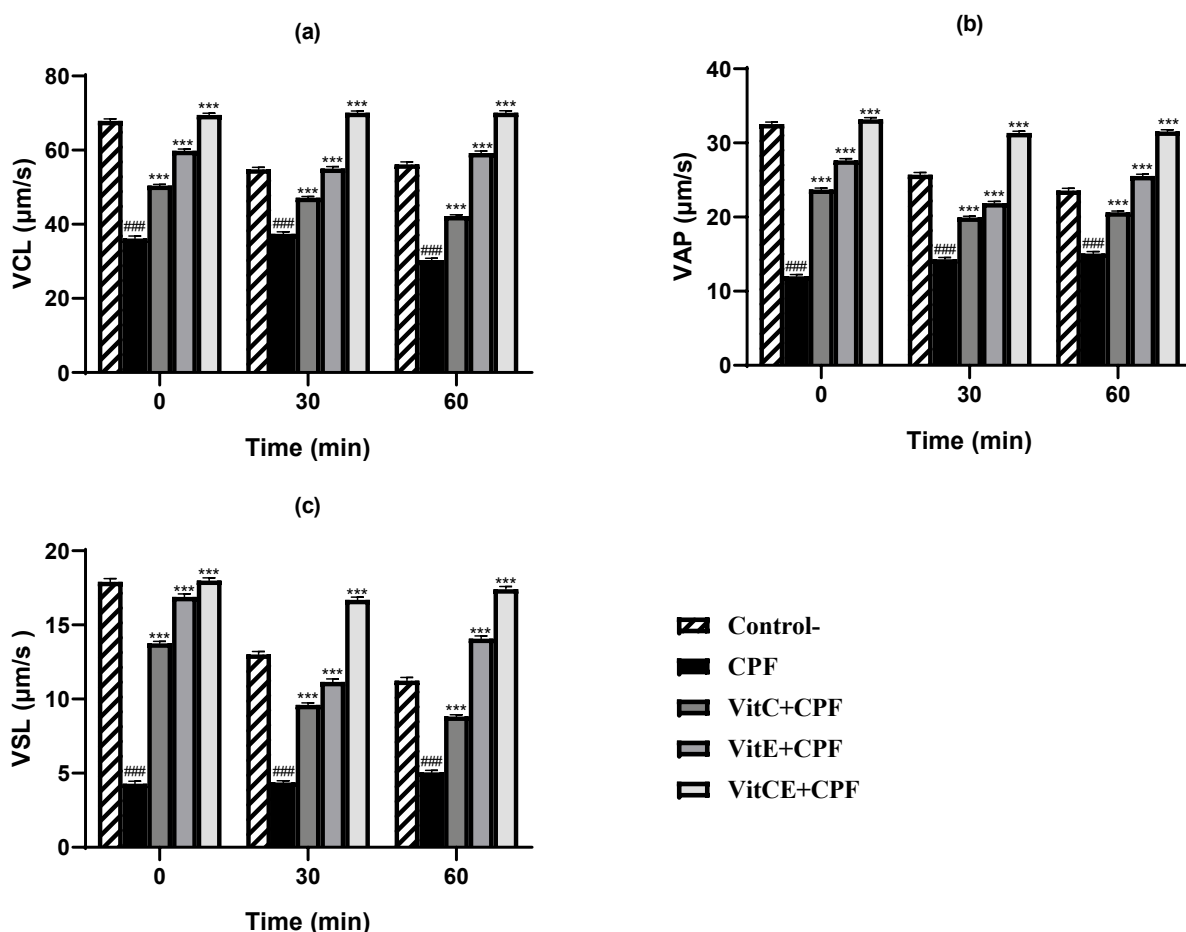


Fig. 1. Kinetic parameters of spermatozoa co-incubated with chlorpyrifos and vitamins. (mean \pm SEM) values for (a) curvilinear velocity (VCL), (b) average path velocity (VAP), (c) straight-line velocity (VSL), at 0, 30, and 60 min in the five tested groups

Significant difference between CPF and Control-. * Significant difference between vitamin-treated groups and CPF. # $P < 0.05$; ## $P < 0.01$; ### $P < 0.001$. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 1. Comparative values (mean \pm SEM) of sperm kinetic parameters at the 60 min evaluation time point

	Control-	CPF	VitC+CPF	VitE+CPF	VitCE+CPF
VCL ($\mu\text{m/s}$)	56.186 \pm 0.676	30.347 \pm 0.507###	42.232 \pm 0.328***	59.236 \pm 0.551***	70.110 \pm 0.522***
VAP ($\mu\text{m/s}$)	23.571 \pm 0.311	15.095 \pm 0.247###	20.641 \pm 0.161***	25.549 \pm 0.245***	31.563 \pm 0.231***
VSL ($\mu\text{m/s}$)	11.248 \pm 0.209	5.051 \pm 0.143###	8.831 \pm 0.112***	14.071 \pm 0.192***	17.405 \pm 0.185***

Significant difference between CPF and Control-. * Significant difference between vitamin-treated groups and CPF. # $P < 0.05$; ## $P < 0.01$; ### $P < 0.001$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

at all time points (0 min: $P = 0.0009$; 30 min: $P = 0.012$; 60 min: $P = 0.032$). The same trend was observed in progressive motility, with a significant reduction only at 0 min ($P = 0.015$). No significant differences were observed at 30 and 60 min.

Among the vitamin-treated groups, the VitCE+CPF group showed higher PM and R values compared with the CPF group. At 60 min, VitCE+CPF showed PM and R values of $9.112 \pm 3.357\%$ and $29.374 \pm 9.379\%$, respectively, compared with $0.225 \pm 0.084\%$ and $1.365 \pm 0.232\%$ in the CPF group. However, these differences were not statistically significant. In contrast, the VitE+CPF group showed a significant

improvement in PM and R compared with the CPF (PM: $P = 0.018$; R: $P = 0.004$). No significant differences were observed for the VitC+CPF group. Mean values are summarized in Table 2.

Lipid Peroxidation

Lipid peroxidation was assessed after 30 min of co-incubation in all groups. As shown in Fig. 3, MDA levels differed significantly among the experimental groups. Compared with the Control- group, MDA levels were significantly higher in the CPF group ($P = 0.035$). Comparative values (mean \pm SEM) are presented in Table 3.

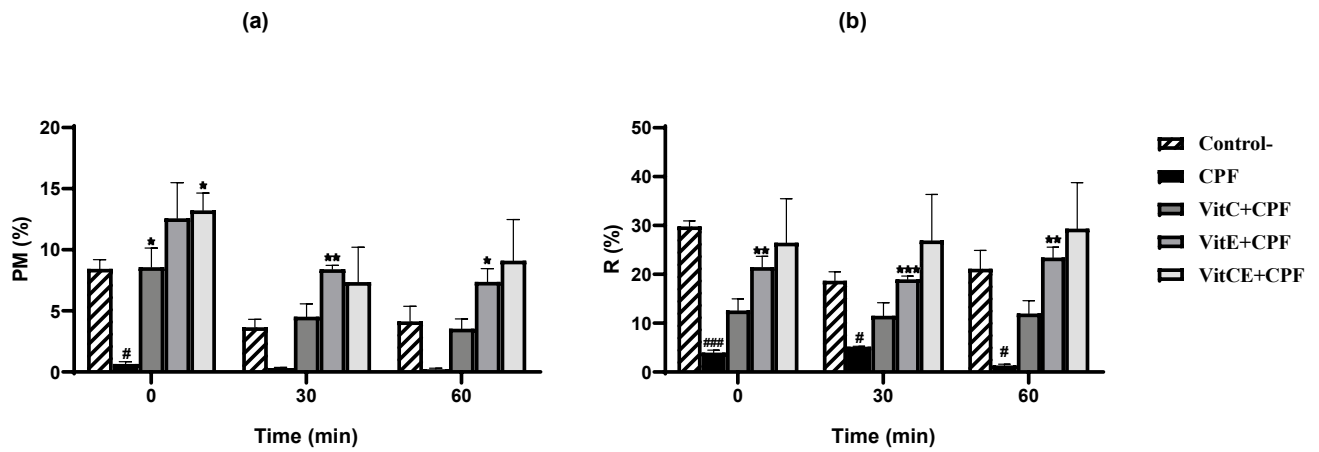


Fig. 2. Percentages (mean ± SEM) of progressive motility (PM) (a) and rapid motility (b) at 0, 30, and 60 min in the five groups

Significant difference between CPF and Control-. * Significant difference between vitamin-treated groups and CPF. # $P < 0.05$; ## $P < 0.01$; ### $P < 0.001$. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Absence of symbols indicates no statistically significant difference from CPF ($P > 0.05$).

Table 2. Comparative values (mean ± SEM) of progressive motility (PM) and rapid motility (R) at the 60 min evaluation time point

	Control-	CPF	VitC+CPF	VitE+CPF	VitCE+CPF
Progressive motility (%)	4.146 ± 1.224	0.225 ± 0.084	3.542 ± 0.807	7.371 ± 1.084*	9.112 ± 3.357
Rapid motility (%)	21.153 ± 3.749	1.365 ± 0.232#	11.967 ± 2.632	23.446 ± 2.134**	29.374 ± 9.379

Significant difference between CPF and Control-. * Significant difference between vitamin-treated groups and CPF. # $P < 0.05$; ## $P < 0.01$; ### $P < 0.001$. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Absence of symbols indicates no statistically significant difference from CPF ($P > 0.05$).

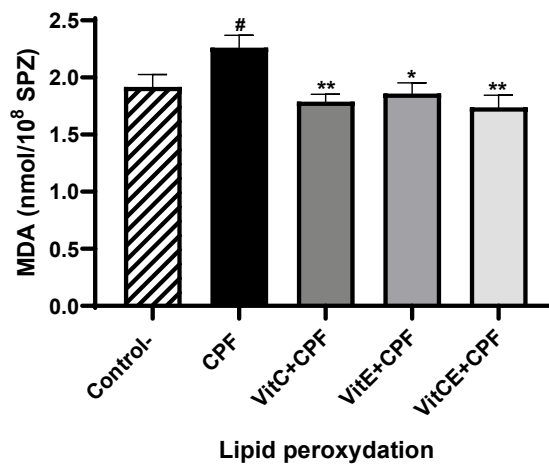


Fig. 3 Malondialdehyde (MDA) levels Values are presented as mean ± SEM.

Significant difference between CPF and Control-. * Significant difference between vitamin-treated groups and CPF. # $P < 0.05$; ## $P < 0.01$; ### $P < 0.001$. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 3. Comparative values (mean ± SEM) of malondialdehyde (MDA) concentration at the 30 min evaluation time point

	Control-	CPF	VitC+CPF	VitE+CPF	VitCE+CPF
MDA levels (nmol MDA/10 ⁸ SPZ)	1.919 ± 0.109	2.261 ± 0.108#	1.788 ± 0.066**	1.860 ± 0.092*	1.739 ± 0.107**

Significant difference between CPF and Control-. * Significant difference between vitamin-treated groups and CPF. # $P < 0.05$; ## $P < 0.01$; ### $P < 0.001$. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

MDA levels were decreased in the vitamin-treated groups, with the lowest value in the VitCE+CPF group (1.739 ± 0.107 nmol MDA/ 10^8 SPZ); compared with the CPF group ($P = 0.003$). Significant differences were noted between VitC+CPF ($P = 0.007$) and VitE+CPF ($P = 0.029$) groups compared with the CPF group.

Discussion and Conclusions

Male reproductive function is susceptible to a broad spectrum of chemicals, particularly pesticides (Ashiru and Odusanya, 2009), which have received significant research attention in recent decades. Several toxicological studies have indicated that chlorpyrifos (CPF) can be harmful to various mammal species, affecting multiple organs and tissues. It has been especially reported that reproductive function was affected at different levels, impairing hormone secretion and testicular integrity (Moreira et al., 2021; Darwish et al., 2025). The use of in vivo models has revealed significant impairment in all sperm parameters. However, it remains unclear whether this impairment is related to hypothalamic-pituitary-gonadal axis dysregulation and testicular impairment or to a direct impact on mature spermatozoa. In this context, the present study aimed to assess the direct in vitro impact of CPF on mature spermatozoa, to evaluate whether oxidative stress may contribute to CPF-induced toxicity as assessed by lipid peroxidation, and to investigate the effects of vitamins C and E, either individually or in association.

Kinetic parameters, including VCL, VAP, and VSL, as well as progressive and rapid motility, were evaluated in all experimental groups. The results showed a notable decrease in all sperm motility parameters in the CPF-treated group, whereas the groups receiving vitamins C and E exhibited higher values than the CPF group, particularly when they were associated. In addition, CPF significantly increased MDA levels, indicating enhanced lipid peroxidation. This increase was reduced in the presence of vitamins C and E or their association. These findings are consistent with in vivo studies reporting that CPF induces complete loss of motility in male mice, along with a concomitant increase in OS (Zhang et al., 2020). Furthermore, CPF has been associated with lipid peroxidation in testicular membranes and oxidative stress production (Mandal and Das, 2011). In fact, OS has been proposed as a key mechanism underlying the toxicities of several pesticides, including organophosphates (Abdollahi et al., 2004). In a comparable in vitro study on caprine testicular cells, Bhardwaj et al. (2018) assessed oxidative stress induced by cypermethrin by measuring lipid peroxidation (TBARS) and ferric reducing antioxidant power (FRAP), alongside antioxidant enzyme activities including catalase (CAT), superoxide dismutase (SOD), and glutathione-S-transferase (GST).

Mammalian sperm cells are highly susceptible to oxidative damage owing to their high content of polyunsaturated fatty acids, which are prone to peroxidation (Sheweita et al., 2005; Wathes et al., 2007; Chianese and Pierantoni, 2021). In this context, docosahexaenoic acid (DHA), a major fatty acid in sperm cell membranes, is especially vulnerable to lipid peroxidation due to its six double bonds (Wright et al., 2014). This can result in elevated production of reactive oxygen species (ROS), leading to increased sperm DNA fragmentation and mitochondrial dysfunction, ultimately contributing to male infertility (Kumar and Singh, 2022). Moreover, spermatozoa contain a large number of mitochondria due to their constant energy requirements for optimal motility (Darmawan, 2007). These mitochondria, as an energy source, are significantly affected by the integrity of the sperm's tail and midpiece (Talwar and Hayatnagarkar, 2015; Chianese and Pierantoni, 2021). In fact, Zhang et al. (2020) have shown a decrease in sperm motility in mice induced by CPF associated with decreased mitochondrial activity.

Several antioxidants, including vitamin C, vitamin E, glutathione, and coenzyme Q10, are recognized for their efficacy in treating male infertility (Sheweita et al., 2005). Regarding the vitamin treatment, results showed that both vitamins C and E were associated with attenuation of the CPF-induced impairment of sperm motility, especially when associated. Previous studies have demonstrated that antioxidant supplementation significantly improves sperm parameters. Indeed, Zalata et al. (2014) reported the protective effects of vitamins C (20 mM) and E (2 mM) on human spermatozoa exposed to cypermethrin. Bhardwaj et al. (2018) have also demonstrated that vitamins C and E at 0.5 and 1.0 mM reduced cytotoxicity and oxidative stress in the testicular tissue of male caprine exposed to cypermethrin. These findings are consistent with our findings.

Vitamin E, a lipophilic antioxidant, acts as a chain-breaking agent in lipid peroxidation by scavenging peroxy and alkoxy radicals, thereby preventing free radical amplification (Hammadeh et al., 2009). Vitamin C (ascorbic acid), a hydrophilic antioxidant, neutralizes free superoxide, peroxy, and hydroxyl radicals (Wefers and Sies, 1988). The difference between vitamin E and vitamin C may be attributed to vitamin E's lipophilic nature, which facilitates its free distribution within cell membranes (Takhshid et al., 2012). The combination of vitamins C and E was associated with more attenuation of CPF-induced toxicity, compared with vitamins alone. This may be related to the capacity of vitamin C to regenerate the oxidized form of vitamin E, thereby optimizing antioxidant defence (Wefers and Sies, 1988).

In conclusion, the current in vitro study demonstrates that chlorpyrifos significantly impairs sperm kinetic parameters and increases lipid peroxidation in mature epididymal spermatozoa. This

suggests that oxidative stress may contribute to CPF-induced sperm toxicity. Pre-treatments with vitamins C and E attenuate these effects, particularly when they are associated.

However, several limitations in the present study should be acknowledged. First, oxidative stress was assessed using only a single marker of lipid peroxidation (MDA). Therefore, future studies should include additional oxidative stress biomarkers, such as SOD, CAT, and GSH activities, to provide a more comprehensive evaluation of oxidative stress involvement. Second, the relatively limited sample size (N = 5) may limit the generalizability of the findings to the broader caprine population. Furthermore, expressing results as mean \pm SEM, while useful for comparing treatment groups, does not fully reflect inter-individual variability. Also, the absence of vitamin-only control groups limits the distinction of

the intrinsic effects of vitamins C and E from CPF-related interactions. Future investigations should involve larger sample sizes, multiple oxidative stress markers, and appropriate vitamin-only control groups to strengthen the current results. In addition, artificial insemination studies could reveal the real interest of such vitamin protection on fertility outputs.

Acknowledgment

The authors express their gratitude to the Ministry of Higher Education and Scientific Research (MESRS) and the Directorate General for Scientific Research and Technological Development (DGRSDT) of Algeria for their financial support. The authors would also like to thank the Faculty of Natural and Life Sciences (SNV) of the University of Béjaïa for their valuable assistance.

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Supplement

The 3rd International scientific online conference “One Health: Challenges for Food Safety”, which was organized by the Lithuanian University of Health Sciences, Veterinary Academy, Department of Food Safety and Quality and Lithuanian Academy of Sciences and took place virtually on 15 April 2026

ONE HEALTH: CHALLENGES FOR FOOD SAFETY

Abstracts

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REMOTE MEAT CONTROL – FLEXIBILITY FOR SUSTAINABILITY

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According to current EU-regulations, live animals before slaughter and carcasses after slaughter must be inspected onsite by official control at commercial establishments. A large part, 80%, of the Swedish slaughterhouses and game handling establishments are up and running only few days per week or seasonally. Swedish Food Agency's (SFA's) control staff drive to and from these places every slaughter day. This means many hours in the car and logistical vulnerability due to, for example, harsh weather conditions or illness. The costs ultimately burden consumer prices. All driving has a high environmental impact by contributing to global emissions.

Since 2019, Swedish University of Agricultural Sciences together with the SFA has studied a model for remote meat control. This means that inspections both ante and postmortem take place with the help of digital transmission of images and sounds instead of onsite in the facility. The goal is to create flexibility for sustainability within the EU by using remote control in small-scale slaughter and game management under well-defined circumstances.

Our results show that the remote method is feasible and does not compromise food safety, animal health, or welfare. The variation in inspection results was greater between different individual control persons than between the methods. The behavioural studies amongst control staff show that the digital security is a central issue. Prerequisites as good food safety culture, well trained staff and healthy animal populations must be in place. Remote meat control has clear positive effects on the environment, the economy, and the work environment.

Keywords: antemortem inspection, domestic ungulates, game, postmortem inspection, risk management.

MICROBIAL FOOD SAFETY: DECONTAMINATION IN THE POULTRY MEAT CHAIN WITH A FOCUS ON CAMPYLOBACTER

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Campylobacteriosis remains the most commonly reported bacterial zoonosis in the European Union. The primary route of transmission is poultry meat, which is frequently contaminated with *Campylobacter* spp. The contamination of the poultry meat occurs during slaughter and processing at the slaughterhouse, particularly through cross-contamination between carcasses or via contaminated surfaces and process water.

Since no method has been established yet to eradicate *Campylobacter* spp. from poultry meat completely, current research is focused on strategies that reduce the bacterial count on the carcasses and thereby lower the risk of human infections. Measures under investigation currently include treatment with UV-C radiation, hard scalding or ultracold air during the slaughter and processing.

Similar to findings from primary production, it is becoming apparent within the poultry slaughter chain that individual measures alone achieve only limited effects. Rather, a combination of multiple intervention strategies along the entire process chain appears to be a promising approach for achieving an effective and sustainable reduction in *Campylobacter* contamination in poultry meat.

Keywords: poultry, slaughter, *Campylobacter*, intervention methods.

FOOD SAFETY ISSUES IN POULTRY MEAT: ANTIBIOTIC RESIDUES AND ALTERNATIVE CONTROL STRATEGIES FOR *SALMONELLA ENTERICA*

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Within the European Union, systematic monitoring of veterinary medicinal product residues in food-producing animals is a regulatory requirement. In this study, in total, 40 poultry meat samples were collected from local markets, across Estonia, Latvia, and Lithuania (the origin countries: Latvia, Lithuania, Estonia, Poland, France). Application of an ultra-sensitive ultra-high performance liquid chromatography–MS/MS analytical approach enabled the detection of enrofloxacin and ciprofloxacin residues, with enrofloxacin identified at trace levels in 97% of poultry meat samples. In the next stage of the experiment, the antimicrobial efficacy of 13 lactic acid bacteria (LAB) strains, 2 essential oils – *Thymus vulgaris* and *Origanum vulgare* L. – and their combined formulations was evaluated for their ability to inhibit antibiotic-resistant *Salmonella* spp. It was established that the most effective formulation for *Salmonella* control comprised thyme essential oil (1.0%) combined with the following LAB strains: *Lb. plantarum* (LUHS122), *Leuc. mesenteroides* (LUHS242), *Lb. casei* (LUHS210), *Lb. paracasei* (LUHS244), *Lb. plantarum* (LUHS135), *Lb. coryniformis* (LUHS71), and *Lb. uvarum* (LUHS245). Notably, the majority of LAB strains exhibited growth inhibition when exposed to thyme essential oil at concentrations of 0.5% and 1.0%, with the exception of strains LUHS122, LUHS210, and LUHS245, which demonstrated tolerance under these conditions. Overall, these findings highlight the need for further investigations aimed at identifying specific LAB-derived metabolites responsible for antimicrobial activity against *Salmonella* spp., thereby enabling more targeted and effective intervention strategies.

Keywords: antimicrobials, lactic acid bacteria, essential oil, pathogens inhibition.

RELATIONSHIP BETWEEN BEEF CARCASS QUALITY AND WELFARE DURING REARING, TRANSPORTATION AND SLAUGHTER IN LITHUANIAN CATTLE FARMS

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Integrated animal welfare assessment systems, such as the Welfare Quality[®] assessment system and the Animal Needs Index (ANI), based on systematic data collection and analysis, provide a more structured and comprehensive approach than the conventional methods currently used in Lithuanian cattle farms. The objective of this study was to determine the correlations between cattle welfare during rearing, transport, and the pre-slaughter period – evaluated using the Welfare Quality and ANI systems – and carcass quality.

Four beef cattle farms (346 cattle) were monitored using these welfare systems in order to identify and eliminate factors negatively affecting animal welfare during rearing, transport, and before slaughter. Limousin cattle were the most sensitive to stress, Charolais cattle were sensitive to a lesser extent, while Aubrac and Highland cattle were the most resistant. The potential for meat maturation depended on carcass conformation class and the pH decline during the first 48 hours postmortem. Only Charolais carcasses were classified as EUROP conformation class U, whereas Limousin and Highland carcasses were classified as R and O classes, respectively. Under appropriate welfare conditions, short transport and acute pre-slaughter stress had no statistically significant effect on beef carcass quality or meat quality parameters.

Keywords: animal welfare, stress, beef carcass, meat quality.

LIMITED GENETIC HOMOGENEITY BETWEEN *SALMONELLA* ENTERITIDIS ISOLATED FROM BROILER CHICKENS AND RETAIL BROILER MEAT

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Salmonella is an important foodborne pathogen which is mostly associated with consumption of poultry meat. The aim of the present study was to analyze the characteristics of *Salmonella* isolates from retail broiler meat and to compare those with the isolates obtained from broiler chickens. Retail samples were collected during 2025. Samples were investigated microbiologically; whole genome sequencing was used for characterization of *Salmonella* isolates. *Salmonella* was isolated from seven retail broiler meat samples, including wings, thighs and drumstick samples. Among seven *Salmonella* positive samples, six contained serovar Enteritidis and one Typhimurium. All Enteritidis isolates belonged to Sequence Type (ST) 11. When comparing isolates from retail meats with available broiler chickens' isolates by cgMLST method, a big cluster of closely matching isolates was retrieved. In total, three distinct subclusters were identified, all containing isolates originating from multiple countries and over multiple years. Since most of the *Salmonella*-positive meat was from Poland but many of the matching poultry isolates were of Latvian origin, the observed similarities indicate genetic homogeneity of *Salmonella* Enteritidis ST11 with limited opportunity to track dissemination sources of serovar Enteritidis in broiler meat production chain in Latvia.

Keywords: *Salmonella*, Latvia, broiler meat, sequence type, Enteritidis.

APPLICATION OF PORCINE LIVER PROTEIN HYDROLYSATES IN DEVELOPMENT OF ANTIOXIDANT AND ANTIMICROBIAL FUNCTIONAL GUMMY CANDIES

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The growing demand for functional foods enriched with natural bioactive compounds has encouraged the exploration of alternative protein sources and the development of value-added products. This study aimed to develop functional gummies enriched with protein hydrolysates derived from porcine liver and to evaluate their antioxidant, antimicrobial, and microbiological properties. The enzymes for hydrolysis were selected based on the degree of hydrolysis (DH), which served as the primary indicator of hydrolytic efficiency; papain and pepsin were chosen for further investigation. Enzyme type, hydrolysis time, and their interaction significantly affected the DH ($P < 0.001$).

The developed gummies were evaluated for sensory acceptability, antioxidant activity (ABTS•+, DPPH•), antimicrobial effects, and microbiological stability over a 21-day storage period. The incorporation of liver hydrolysates significantly enhanced antioxidant activity ($P \leq 0.05$). The highest radical scavenging capacity was observed in the GC5Pa24Ag sample (papain, 24 h, agar), reaching $67.6 \pm 0.98 \mu\text{mol/g}$ (ABTS•+) and $49.14 \pm 1.00\%$ (DPPH• inhibition). During storage, gelatin-based gummies showed a gradual increase in bacterial counts (up to $2.86 \log_{10}$ CFU/g), while agar-based formulations remained microbiologically stable throughout the study period.

Keywords: porcine liver protein hydrolysates, functional gummies, antioxidant activity, antimicrobial properties.

FUNCTIONALITY AND SENSORY PROPERTIES OF CHICKEN LIVER PÂTÉS ENRICHED WITH OMEGA-3 FATTY ACID SOURCES IN TANDEM WITH PLANT-BASED ADDITIVES

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The development of functional food products enriched with polyunsaturated fatty acids is an essential strategy for cardiovascular disease prevention, as these conditions remain the leading cause of death worldwide. This study aimed to develop recipes for chicken liver pâtés enriched with omega-3 fatty acid sources (flaxseed and rapeseed oils) combined with various antioxidant additives: walnuts, dried cranberries, dried plums, and orange peel. The study objective was to evaluate the sensory properties and functionality of the developed products, specifically focusing on the omega-6/omega-3 fatty acid ratio and the atherogenicity (AI) and thrombogenicity (TI) indices.

Original recipes for both baked and spreadable chicken liver pâtés were developed. Fatty acid (FA) profiles and omega-6/omega-3 ratios were determined using gas chromatography GC-MS method, and AI and TI indices were calculated. While all additives positively influenced the fatty acid profile and functionality, the most significant improvements were observed in samples containing walnuts and flaxseed oil. These products showed increased levels of polyunsaturated FAs, reduced saturated FAs, and enhanced overall nutritional value, compared with the control. Furthermore, the addition of walnuts and flaxseed oil improved the intensity of taste and aroma, reduced sourness, and increased general consumer acceptance.

Keywords: chicken liver pâté, fatty acids profile, functionality, atherogenicity.

IMPACT OF BIOPRESERVATION ON THE ACCUMULATION OF CARCINOGENIC SUBSTANCES IN HEAT-TREATED PORK PRODUCTS

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The continued use of synthetic additives in the meat industry remains controversial due to their potential carcinogenicity. Therefore, the purpose of this research was to evaluate the effect of selected biopreservatives on the accumulation of polycyclic aromatic hydrocarbons (PAHs) and N-nitrosamines in hot-smoked pork sausages and grilled pork shashlik. The hot-smoked sausages were produced in a small-scale meat processing facility. The study included physico-chemical and sensory evaluations. PAH content was determined using liquid chromatography, while volatile N-nitrosamines were quantified via gas chromatography-mass spectrometry (PerkinElmer Clarus 680). The results demonstrated that in hot-smoked sausages, a mixture of rosemary and pomegranate extracts, green tea extract, and rosemary extract combined with *S. carnosus* and *S. vitulinus* bacterial cultures significantly reduced nitrite and total N-nitrosamine levels ($P < 0.05$) compared with the control. In grilled pork shashlik, *L. plantarum* MI-LP1 was identified as the most effective culture for reducing the accumulation of both biogenic amines and N-nitrosamines ($P < 0.05$). Furthermore, lower cooking temperatures ($< 180^{\circ}\text{C}$) resulted in significantly lower volatile N-nitrosamine levels ($P < 0.05$).

Keywords: pork, biopreservation, N-nitrosamines, polycyclic aromatic hydrocarbons.

MICROBIOLOGICAL FOOD SAFETY AND QUALITY INDICATORS OF MINCED MEAT

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Meat is one of the most popular food sources in the human diet in today's age. The produce is a constant subject to contamination with microorganisms from farm to fork. A common pathogen for the contamination of minced meat is *Escherichia coli*. Studies have shown a possible prevalence of *E. coli* from retail ground beef of up to 55%. Furthermore, *E. coli* has been proven to be resistant to certain antimicrobial agents. This is posing a significant challenge to the food safety, quality and the public health. To prevent and identify contamination, food quality and safety indicators including aerobic colony count and *E. coli* number can be used.

In total, 20 minced meat samples have been collected from various food distributors in Lithuania. Aerobic colony count requirement was not met in 35% of the samples (EC) No (1441/2007). *E. coli* was also found in 35% of the minced meat samples. An antimicrobial resistance study, by determining the minimum inhibitory concentration (MIC) of *E. coli* growth, using the Liofilchem[®] E test strips with 14 different antimicrobial agents, was performed. All tested *E. coli* isolates were susceptible to F, CRO, MRP, IMI, FOS, CN, ATM and AK. Partial resistance occurred in PX, CIP, SAM, AMC, SXT and TE, with percentages ranging from 12.5% up to 87.5%. In total, 37.5% of *E. coli* isolates were confirmed as multi-drug resistant.

Keywords: minced meat, aerobic colony count, *E. coli*, antimicrobial resistance.

MICROBIAL COMMUNITIES AND CIRCULATION OF ANTIMICROBIAL RESISTANCE GENES IN BROILER FARMS

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Antimicrobial resistance is a growing concern in poultry production and food safety, where complex microbial communities can influence the persistence and spread of resistance determinants. Poultry farms harbour diverse microbiota that may affect both the development of broiler gut microbiota and the circulation of antimicrobial resistance within farm environments.

In this study, shotgun metagenomic sequencing was used to analyse microbial communities and antimicrobial resistance genes in broiler faecal and farm environmental samples, including feeders, drinkers and boot swabs, collected throughout the broiler growing period.

Metagenomic analysis revealed differences between environmental and faecal microbial communities. Environmental samples were mainly dominated by Proteobacteria. In contrast, the composition of faecal microbiota changed during broiler growth. In the early stage of the growing period, Proteobacteria were more abundant in the broiler gut microbiota. In contrast, Firmicutes gradually increased and became dominant in the later stage, reflecting the maturation of the intestinal microbiota.

Antimicrobial resistance genes were detected throughout the broiler growing period. β -lactam resistance genes, including *blaCTX-M* and *blaTEM*, were particularly common during the early stage of broiler growth. In later stages, tetracycline resistance genes (*tetA*, *tetB*) and sulphonamide resistance genes (*sul1*, *sul2*) were detected more frequently. Mutations associated with quinolone resistance (*gyrA*, *parC*) were detected at multiple stages of broiler growth.

Although the composition of microbial communities changed substantially during broiler growth, antimicrobial resistance determinants were detected throughout the entire growing period. This indicates that resistance genes may persist within the broiler farm microbiome despite changes in bacterial community composition.

Keywords: metagenomics, antimicrobial resistance, broilers, microbiome, poultry farm.

ANTIMICROBIAL SUSCEPTIBILITY OF *SALMONELLA* SPP. ISOLATES FROM PORK SLAUGHTERHOUSES IN PORTUGAL: A ONE HEALTH CONTRIBUTION

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Antimicrobial resistance (AMR) is widely recognized as one of the most pressing global health challenges, requiring coordinated surveillance across human, animal and environmental domains. *Salmonella* spp. remains a leading cause of foodborne illness in the European Union, with pork products consistently identified as one of the major sources of human infection. However, systematic data characterizing antimicrobial susceptibility patterns of *Salmonella* spp. at slaughterhouse level in Portugal remain limited, even though slaughterhouses constitute a critical interface within the food production continuum.

This study assesses the occurrence of *Salmonella* spp. in food samples from Portuguese pork slaughterhouses and integrates microbiological and process data to identify critical points driving the transmission of antimicrobial-resistant strains.

Three pork slaughterhouses located in the central region of Portugal were selected based on production capacity, operational representativeness and geographical distribution. Carcass sampling for microbiological testing was performed in different processing stages. Microbiological evaluation for process hygiene and safety indicators was performed according to international standards. Potential dissemination pathways within the processing environment of *Salmonella* spp. will be further explored, together with the isolates' antimicrobial susceptibility.

Taken together, our results are expected to facilitate the effective implementation of the One Health paradigm within food safety systems and, ultimately, strengthen national AMR surveillance capacity.

Keywords: *Salmonella* spp., pork, slaughterhouse, antimicrobial resistance.

EVALUATING THE SPOILAGE POTENTIAL OF *VAGOCOCCUS* SPP. IN MODIFIED ATMOSPHERE PACKAGED BROILER

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Microbial spoilage causes significant economic losses in the meat industry. While diverse bacteria contribute to this process, *Vagococcus fessus* and *Vagococcus proximus* have emerged as potential key spoilers. This study investigates their specific impact using streptomycin-resistant strains to track growth within the natural microbiota of commercial broiler chicken.

Six *Vagococcus* strains were prepared as two distinct cocktails and inoculated into chicken packages, alongside uninoculated controls, then stored at 6°C for 14 days. Growth was quantified on selective media, while spoilage was assessed via sensory analysis (odor, color, texture) and volatile organic compound (VOC) profiling using gas chromatography (GC).

Preliminary trials with wild-type *V. proximus* (PNs007T) in modified atmosphere packaging showed media-specific growth, with TSB and PCA supporting higher densities than MRS or Elliker media. These dynamics suggest that nutrient availability significantly influences metabolic activity. We aim to correlate specific VOC signatures and sensory changes directly to *Vagococcus* activity, distinguishing their contribution from background flora.

By integrating microbiological and sensory analyses, this research clarifies the role of *Vagococcus* spp. in meat decay, providing essential data to improve shelf-life and food safety strategies.

A ONE HEALTH CONCEPTUAL FRAMEWORK FOR FOOD SAFETY MANAGEMENT IN THE *CAMELUS DROMEDARIUS* MEAT PRODUCTION CHAIN

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Camel meat production represents an essential source of animal protein in arid and semi-arid regions, where *Camelus dromedarius* contributes significantly to food security and rural economies. Despite its importance, systemic approaches integrating the entire meat production continuum remain limited for this species. Food safety management is often addressed at isolated stages, particularly at slaughter level, without fully considering upstream and environmental determinants of contamination.

This study proposes a One Health conceptual framework for food safety management across the *Camelus dromedarius* meat production chain. The model adopts a holistic perspective integrating primary production, animal health status, environmental exposure, transport conditions, slaughter hygiene, and post-slaughter conservation. At farm level, factors such as water quality, feeding practices, and extensive breeding systems may influence microbial carriage and baseline risk profiles. Environmental stressors, particularly thermal stress typical of desert ecosystems, may affect animal physiology, immune response, and subsequent meat quality parameters, including pH evolution and microbial ecology.

Transport and slaughtering stages are identified as critical risk interfaces where stress-induced microbial shedding and cross-contamination may occur. Post-slaughter handling, including temperature control and storage management, further determines microbial stability and spoilage dynamics.

This framework is proposed as a structured scientific basis to guide future experimental research, risk assessment strategies, and coordinated safety governance in camel meat production systems.

Keywords: camel meat, food safety, one health, conceptual framework, meat production chain, risk management.

INVISIBLE BIOLOGICAL AND SYSTEMIC THREATS IN MEAT FOOD SAFETY: A ONE HEALTH ASSESSMENT OF AZERBAIJAN'S PRODUCTION CHAIN

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The meat production chain constitutes a complex biological and environmental interface where microbiological hazards, chemical contaminants, and antimicrobial resistance (AMR) may emerge and disseminate. In Azerbaijan, increasing livestock intensification and modernization of meat processing systems necessitate a comprehensive risk-based evaluation of food safety threats. This study applies to a One Health framework to assess invisible biological and systemic risks within the national production chain.

Primary production has been identified as the critical control stage for hazard introduction. Regional evidence indicates seasonal variation in organochlorine (α - and δ -HCH) and pyrethroid pesticide residues in bovine and ovine tissues. Although organochlorines are largely restricted, environmental persistence contributes to occasional detection. Certain pyrethroid compounds, including lambda-cyhalothrin and bifenthrin, have been reported at concentrations exceeding established maximum residue limits in comparable contexts, underscoring the importance of systematic residue monitoring and regulatory enforcement.

Concurrently, antimicrobial misuse in livestock production facilitates the emergence of resistant bacterial strains capable of entering the food chain. The transmission of antimicrobial-resistant pathogens across the human–animal–environment interface increases therapeutic challenges, healthcare costs, and public health risks. Without coordinated intervention, AMR may compromise both veterinary and clinical treatment efficacy.

Strengthening Azerbaijan's meat safety system requires integrated veterinary surveillance, laboratory capacity enhancement, residue control programs, hygienic modernization of slaughterhouses, and responsible antimicrobial stewardship. Harmonization with international standards, including Codex Alimentarius guidelines, is essential. Adoption of a coordinated One Health strategy will enhance resilience, sustainability, and public health protection within Azerbaijan's meat production sector.

Keywords: meat safety, one health antimicrobial resistance, pesticide residues, Azerbaijan, food chain surveillance.

CORRELATION BETWEEN VIRULENCE GENES AND ANTIMICROBIAL RESISTANCE IN *SALMONELLA* SEROVARS ISOLATED FROM MEATS IN ALGERIA

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Within the One Health framework, understanding the link between bacterial virulence and antimicrobial resistance is crucial for food safety. This study specifically aimed to investigate statistical correlations between the presence of virulence genes and antimicrobial resistance profiles in *Salmonella* strains isolated from meats. A total of 84 *Salmonella* isolates belonging to 10 serovars, recovered from bovine and ovine carcasses and feces in Algiers slaughterhouses, were screened by PCR for eight virulence genes (*invA*, *pefA*, *sefA*, *pipB*, *sseC*, *ssaP*, *spvC*, *iroB*). Antimicrobial susceptibility patterns were previously determined. Correlation analyses (Chi-square, Fisher's exact test, Spearman rank correlation) revealed significant associations. Plasmid-borne *pefA* and *spvC* genes, detected only in *S. Typhimurium* (3.6% of isolates), showed strong-to-perfect positive correlations with resistance to tetracycline ($r = 1.000$; $P < 0.001$), chloramphenicol ($r = 0.811$; $P < 0.001$), and penicillins ($r = 0.412$; $P = 0.002$). Conversely, negative correlations were observed between SPI-encoded genes (*invA*, *sseC*, *ssaP*, *pipB*) and resistance to gentamicin ($r = -0.283$; $P = 0.025$) and mecillinam ($r = -0.285$; $P = 0.037$). No significant correlation was found with quinolones. These findings demonstrate that specific virulence determinants are significantly associated with antimicrobial resistance, particularly in *S. Typhimurium* strains exhibiting multidrug resistance (ACSSuT profile). Such associations highlight the potential co-selection of virulence and resistance, posing an enhanced public health risk. This study underscores the need for integrated surveillance along the meat production chain within a coordinated One Health approach to mitigate the spread of high-risk clones.

Keywords: *Salmonella*, meat, virulence genes, antimicrobial resistance, correlation, one health, Algeria.

BOTULISM IN UKRAINE: CAUSES AND CONSEQUENCES

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In Ukraine, approximately 120 cases of botulism are registered annually, 5% of which are fatal. In fact, Ukraine has the worst situation compared with European Union countries.

Due to Russian military aggression, massive destruction of infrastructure, and the suspension of planned state supervision (control) measures for market operators, especially with the onset of the summer season, there has been an increased risk of complications in the sanitary and epidemiological situation regarding acute intestinal infectious diseases and food poisoning.

The extremely unfavorable epidemiological situation with regard to botulism, which has persisted for many years in Ukraine, clearly demonstrates the existing significant shortcomings in both food quality and safety control and public catering establishments. This has been facilitated by the almost complete elimination of control and supervisory functions by the competent authorities: the legislative introduction of a moratorium on inspections of any small and medium-sized businesses, the de facto elimination of the State Sanitary and Epidemiological Service, which supervised the activities of economic entities, as well as insufficient attention to anti-epidemic work at the local level. As practice shows, the issue of biological safety and biological protection of the population requires permanent control over compliance with technological and sanitary regimes for food production at food enterprises, constant control over the rules for long-term storage of meat and fish products in production, widespread anti-epidemic propaganda among the population, etc.

Keywords: botulism, food products.

SAFETY AND QUALITY OF MINCED MEAT IN THE CZECH REPUBLIC

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Minced meat is highly susceptible to microbial spoilage due to its high nutrient content and elevated water activity, and the mincing process further increases microbial load. This study aimed to evaluate the microbiological quality of minced meat of Czech origin and imported products, with a focus on total viable counts (TVC) and *Escherichia coli*. A total of 50 samples of minced meat were analysed, including 30 Czech and 20 imported minced meat samples. TVC and *E. coli* counts were determined according to valid ISO standards. The results showed that imported minced meat had significantly higher TVC ($7.43 \pm 0.78 \log_{10}$ CFU/g) compared with Czech products ($5.66 \pm 1.04 \log_{10}$ CFU/g; $P < 0.001$), indicating a markedly higher level of overall microbial contamination in imported products. The mean *E. coli* count in Czech minced meat was $1.81 \pm 0.75 \log_{10}$ CFU/g, whereas imported products showed a slightly lower mean value of $1.57 \pm 0.44 \log_{10}$ CFU/g. However, this difference was not statistically significant ($P = 0.182$). Overall, the findings demonstrate a significantly higher total microbial contamination in imported minced meat, while the levels of *E. coli* were comparable between Czech and imported products. These results highlight the importance of regular microbiological monitoring of minced meat to ensure food safety and maintain product quality.

Keywords: total viable count, *Escherichia coli*, microbiological quality, meat products.