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Research article

SEASONAL VARIABILITY IN SIMULATED CEPHAPIRIN USE AT DRY-OFF AND ASSOCIATED ENVIRONMENTAL AND ECONOMIC IMPACTS IN HOLSTEIN AND SIMMENTAL COWS

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The study aimed to assess the potential to reduce antibiotic usage during the dry period of dairy cows and the associated environmental impacts by simulating potential cephapirin (Cefa-Safe) application according to the recording season and udder health status of the dairy cow population in Croatia. Official milk recording data from the Croatian Agency for Agriculture and Food (HAPIH) collected between 2013 and 2022 were used for the analysis. Based on these data, cows were classified according to udder health status: healthy (<200000 cells/mL), subclinical mastitis (200000–400000 cells/mL), and clinical mastitis (>400000 cells/mL).

Using these proportions, the total amount of cephapirin, its economic value, and potential environmental emission under the standard blanket dry cow therapy (BDCT) scenario were simulated. The analysis was conducted by season (spring, summer, autumn, winter). The highest estimated antibiotic usage and emissions were observed during summer in Holstein cows and in spring in Simmental cows, whereas the lowest values were recorded in winter for both breeds.

The calculated risk quotients (RQ = PEC/PNEC) exceeded the threshold value RQ > 1 in all seasons, indicating a persistent environmental risk. The economic estimations followed the same pattern, with the highest costs recorded during the warmer part of the year.

The results confirm that seasonal variation affects udder health status and consequently the amount of antibiotics potentially required during the dry period. The proposed model enables a quantitative assessment of the potential for reducing antibiotic usage and provides a scientific basis for transitioning from standard to selective dry cow therapy (SDCT) within the framework of sustainable dairy production.

Keywords: antibiotic emission to the environment, cephapirin, mastitis, season, selective dry cow therapy

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INTRODUCTION

Mastitis remains the most common disease of dairy cows and one of the main causes of economic losses in milk production. The disease leads to reduced milk yield, increased somatic cell count, impaired milk quality, and premature culling of animals [1,2]. In Croatia, similar trends were observed, with mastitis remaining a major cause of economic losses and antibiotic use in dairy herds [3]. The overall economic impact includes direct treatment costs and production losses, but also indirect consequences such as reduced reproductive performance and long-term herd productivity [4]. Parity is another key biological factor influencing mastitis prevalence and milk yield, as higher parity cows show a greater incidence of both subclinical and clinical mastitis [5]. Therefore, strategies for the treatment and prevention of mastitis, especially during the dry period, are crucial for the sustainability of the dairy sector.

The dry period is a crucial phase in which mammary tissue is restored and the risk of new infections is reduced. Dry cow therapy (DCT) was developed to treat existing intramammary infections and prevent new ones during the dry period [2]. Among the predominant pathogens, *Staphylococcus aureus* remains a major cause of chronic intramammary infections in dairy cows [6]. The most commonly used antibiotics at this stage belong to the β-lactam group, among which cephapirin has been shown to be effective due to its rapid metabolism to desacetyl-cephapirin and long-term retention above the minimum inhibitory concentration (MIC) for the most important pathogens [7]. However, the traditional approach of blanket dry cow therapy (BDCT), in which all cows are treated with antibiotics regardless of health status, has led to a significant increase in the total consumption of antibiotics in the dairy sector [8,9]. Such a practice increases the risk of the development of antimicrobial resistance (AMR) and unnecessary exposure of healthy animals to antimicrobial agents [10].

The persistence of environmental bacterial species further complicates mastitis control and links antimicrobial use with farm hygiene and environmental management [11]. Selective dry cow therapy (SDCT) has been introduced as a viable alternative to BDCT, whereby antibiotics are administered only to animals with confirmed intramammary infection, while mechanical barriers such as internal teat sealants are used in healthy cows [12-14]. The results of numerous studies confirm that SDCT allows for a significant reduction in antibiotic use without worsening udder health status [15,16]. Economic analyses have shown that the selective approach reduces treatment costs and overall veterinary drug consumption, especially on farms with a lower prevalence of mastitis [17,18].

In addition to production and health factors, the season plays an important role in the occurrence of mastitis and the effectiveness of therapy. Heat stress, increased humidity, and microclimatic changes during warmer months are associated with higher somatic cell counts and more frequent intramammary infections [19-22]. In summer, there is a decrease in milk yield and increased udder susceptibility, while winter conditions are generally more stable and associated with a lower mastitis prevalence

[23,24]. Such seasonal variations not only reflect the physiological responses of cows to heat stress, but also changes in feeding practices, environmental microbial load, and bedding quality [25,26].

In parallel with the biological aspects, there is growing evidence of the environmental consequences of antibiotic use. A large proportion of the administered doses is excreted unchanged through feces or urine, thus entering soil and aquatic ecosystems [27,28]. Cephapirin and related β-lactam antibiotics can be detected in surface waters at concentrations of up to a few ng/L, and their degradation products are often more chemically stable and sometimes more toxic than the parent compound [29,30]. Environmental risk is assessed by the ratio between the predicted environmental concentration (PEC) and the predicted no-effect concentration (PNEC), with values above 1 indicating a potential hazard to ecosystems and the development of resistance [31]. Such findings further emphasize the importance of rational antibiotic management through seasonally adjusted and selective treatment protocols.

Given the pronounced seasonal variability in udder health status and mastitis incidence, the assessment of the potential for reducing antibiotic use must take into account differences between seasons [19-21,23,32]. The actual udder health structure, based on somatic cell count (SCC) data from official milk recording, allows for the simulation of antibiotic use scenarios by season and breed, since somatic cell count reliably reflects udder health status and the presence of intramammary infections [1,2,25,26].

The aim of the study was to analyse the use of cephapirin as a representative antibiotic during the dry period of Holstein and Simmental cows, with a focus on seasonal variability in the amount of active substance administered, as well as its economic value and potential environmental impact. This approach builds on previous findings regarding the environmental effects and the need for rational antibiotic use in dry cow therapy [8,14,31]. The results could contribute to the existing body of evidence confirming that selective dry cow therapy (SDCT) enables a reduction in antibiotic use without compromising udder health, while simultaneously reducing the environmental load of veterinary pharmaceuticals [8,13,14,31].

MATERIAL AND METHODS

The analysis was conducted using aggregated records from the national milk recording system of cows included in breeding and selection programs in Croatia (HAPIH) for the period from 1 January 2013 to 31 December 2022. Sampling and analysis were performed in accordance with ICAR standards (in the Republic of Croatia, AT4/BT4 testing methods with mathematical correction to the reference A4 method, SLKM HAPIH, accredited laboratory procedures). These records represent cumulative entries over ten years, including multiple lactations of the same animals. After logical data verification (removal of records with extreme or implausible values of production and reproductive parameters), a total of 307531 Holstein and 383208 Simmental cows in

production were included in the analysis. The current national population under milk recording is 20,520 Holstein and 27,040 Simmental cows [33].

The dataset used in this study was the same as in our previous publication [34]. However, while the previous study focused on variability by parity and production level, the present study specifically aimed to examine the seasonal variability in cephapirin use at dry-off in Holstein and Simmental cows, considering udder health status. This study further incorporates scenario-based simulations of antibiotic use per cow per lactation, allowing assessment of ecological and economic implications across seasons.

The data were grouped according to the season in which the last milk recording before the dry period was carried out, namely autumn, winter, spring, and summer. Udder health status at the last milk recording before drying-off was classified according to SCC: healthy cows had <200000 cells/mL, subclinical mastitis 200000–400000 cells/mL, and clinical mastitis >400000 cells/mL [35].

The subject of the assessment was the intramammary administration of Cefa-Safe (active substance: cephapirin) at drying-off. The standard dose per cow was 300 mg per quarter × 4, i.e. 1.2 g of cephapirin. The total mass of the active substance in each group was calculated according to the formula:

$$\text{Amount (g)} = \text{number of treated cows} \times 1.2\ \text{g}$$

Here, 1.2 g of cephapirin represents the standard dose administered per cow per lactation at drying-off. The calculations reflect the simulated antibiotic use per cow per lactation, rather than actual on-farm administration or annual emissions.

The economic value was estimated based on the market offer of the supplier [37], where a package of Cefa-Safe (20 syringes × 300 mg) contains 6 g of cephapirin at a price of €127.64, resulting in a unit price of €21.27 per gram. The seasonal value was calculated according to the formula:

$$\text{Value (€)} = \text{Amount (g)} \times €21.27/\text{g}$$

The potential environmental emission was estimated using a scenario assuming that 60% of the administered dose is excreted in active form via urine and feces, which subsequently enters the environment with manure. This proportion represents a conservative midpoint within the 40–80% range reported in the literature [27, 28]. The emission was calculated as:

$$\text{Emission (g)} = \text{Amount (g)} \times 0.60$$

For an indicative risk assessment, a simplified PEC/PNEC approach was applied, in which the predicted environmental concentration (PEC) was derived by dividing the estimated antibiotic emission (g) by 1000 to obtain values expressed in μg/L as a simplified comparative indicator of potential environmental load, while the predicted no-effect concentration (PNEC) for cephapirin was conservatively set according to literature data and established approaches for environmental resistance thresholds [10,31,38].

The low MIC values of cephapirin against the major mastitis pathogens are consistent with the use of a conservative PNEC threshold [38,39], while pharmacokinetic data indicate rapid metabolism to desacetyl-cephapirin with prolonged retention of concentrations in milk above the MIC level [7].

The risk quotient (RQ) was calculated according to the formula:

RQ = PEC (predicted environmental concentration) / PNEC (predicted no-effect concentration).

The results represent statistical estimates and scenario-based simulations of potential antibiotic use in the blanket dry cow therapy approach, based on the standard dose per cow per lactation (1.2 g of cephapirin). They are presented cumulatively over the ten-year period and reflect simulated use per cow per lactation, rather than actual on-farm administration or annual emissions. All calculations were performed descriptively (sums and proportions by category), while data verification, variable derivation, and aggregation were carried out in SAS 9.4 [36]. Graphical visualizations were prepared based on the same aggregated results using Microsoft Excel.

RESULTS

The udder health status of Holstein cows before dry-off is presented by season in Table 1. During the observed period, the highest proportion of healthy cows (<200,000 cells/mL) was recorded in spring (57.76%), while the lowest proportion was observed in autumn (53.00%). Subclinical mastitis (200,000–400,000 cells / mL) showed a relatively uniform prevalence among seasons, ranging from 17.33% in summer to 19.31% in autumn. Clinical mastitis (>400,000 cells /mL) was most frequent during autumn (27.69%), and least frequent in spring (24.23%). The total estimated amount of cephapirin (Cefa-Safe) administered at dry-off in Holstein cows was 369037.2 g during the entire period. The highest seasonal amount was simulated for summer (109014 g), while the lowest was recorded in winter (78025.2 g).

Table 1. Health status of Holstein cows and total amount of cephapirin (Cefa-Safe) at dry-off by season.

Season	Last milk recording-Cefa-Safe									Total		
	Healthy			Subclinical mastitis			Clinical mastitis					
	N	Amount	%	N	Amount	%	N	Amount	%	N	Amount	%
Autumn	39981	47977.2	53.00	14566	17479.2	19.31	20884	25060.8	27.69	75431	90517.2	100
Winter	34883	41859.6	53.65	12535	15042	19.28	17603	21123.6	27.07	65021	78025.2	100
Spring	44033	52836.6	57.76	13728	16473.6	18.01	18473	22167.6	24.23	76234	91480.8	100
Summer	51304	61564.8	56.47	15742	18890.4	17.33	23799	28558.8	26.2	90845	109014.0	100
Total	**170201**	**204241.2**	**55.34**	**56571**	**67885.2**	**18.40**	**80759**	**96910.8**	**26.26**	**307531**	**369037.2**	**100**

Health status was determined at the last milk recording before dry-off: healthy (<200000 SCC/mL), subclinical mastitis (200000–400000 SCC/mL), and clinical mastitis (>400000 SCC/mL). Amounts were calculated as number of treated cows × 1.2 g cephapirin per cow.

In the Simmental breed, the distribution of udder health categories and the corresponding quantities of cephapirin are shown in Table 2. The highest proportion of healthy cows was observed in spring (59.16%), and the lowest in autumn (52.61%). The proportion of cows with subclinical mastitis ranged from 17.33% in summer to 19.62% in autumn, while clinical mastitis was most frequent in autumn (27.78%) and least common in spring (22.87%). The total estimated amount of cephapirin applied during the dry-off period in Simmental cows was 459849.6 g. The highest seasonal value was simulated in spring (127370.4 g), and the lowest in winter (100360.8 g).

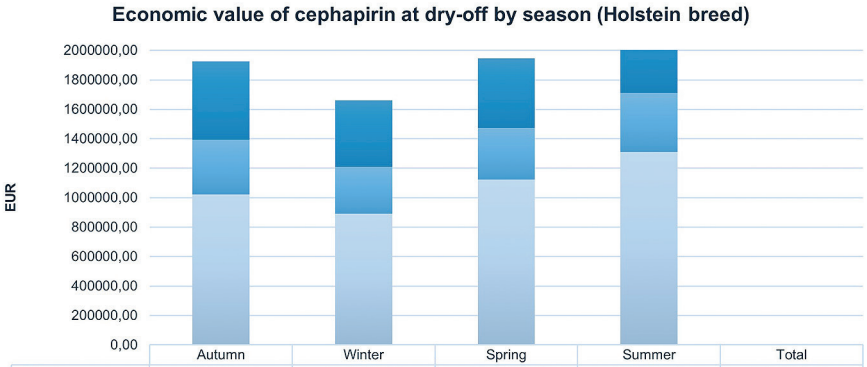
Table 2. Health status of Simmental cows and total amount of cephapirin (Cefa-Safe) at dry-off by season.

Season	Last milk recording-Cefa-Safe									Total		
	Healthy			Subclinical mastitis			Clinical mastitis					
	N	Amount	%	N	Amount	%	N	Amount	%	N	Amount	%
Autumn	48033	57639.6	52.61	17909	21490.8	19.62	25359	30430.8	27.78	91301	109561.2	100
Winter	45251	54301.2	54.11	16337	19604.4	19.53	22046	26455.2	26.36	83634	100360.8	100
Spring	62798	75357.6	59.16	19072	22886.4	17.97	24272	29126.4	22.87	106142	127370.4	100
Summer	57857	69428.4	56.65	17697	21236.4	17.33	26577	31892.4	26.02	102131	122557.2	100
Total	**213939**	**256726.8**	**55.83**	**71015**	**85218**	**18.53**	**98254**	**117904.8**	**25.64**	**383208**	**459849.6**	**100**

Health status was determined at the last milk recording before dry-off: healthy (<200000 SCC/mL), subclinical mastitis (200000–400000 SCC/mL), and clinical mastitis (>400000 SCC/mL). Amounts were calculated as number of treated cows × 1.2 g cephapirin per cow.

The analysis of both breeds indicated that season had a clear effect on the distribution of health categories and the corresponding amount of simulated antibiotic use. In both populations, the highest total quantities of cephapirin were observed in the warmer part of the year (spring and summer), whereas the lowest were estimated for winter. Overall, the proportion of healthy cows was slightly higher in the Simmental breed (55.83%) than in Holstein (55.34%), while the proportions of subclinical and clinical mastitis showed similar seasonal patterns.

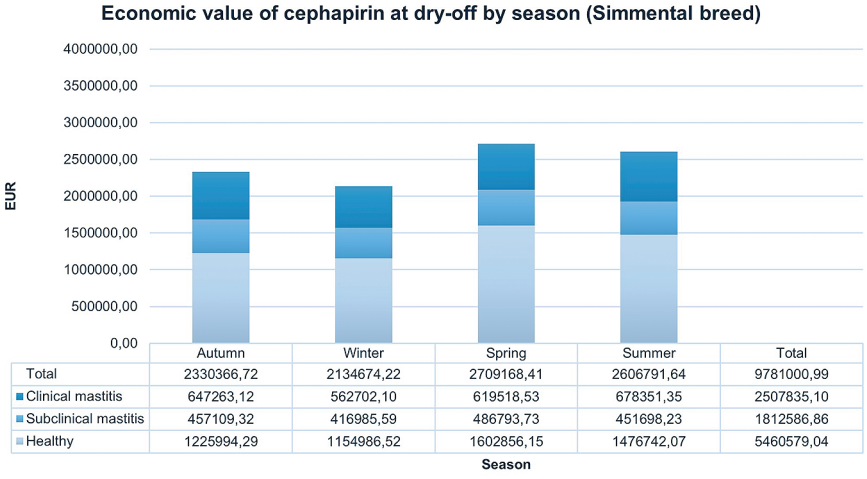
The estimated economic value of the intramammary administration of cephapirin (Cefa-Safe) at dry-off in Holstein cows is presented in Figure 1. The total simulated value across all seasons amounted to €7849421.24, with the highest seasonal value recorded in summer (€2318727.78) and the lowest in winter (€1659596.00). The share of healthy cows in the total value was consistently the highest across all seasons, ranging from 53.0% (autumn) to 57.8% (spring), while subclinical and clinical mastitis together accounted for 42–47% of the total cost. The greatest seasonal reduction in value compared with autumn was observed in winter, reflecting the lower mastitis prevalence during that period.



	Autumn	Winter	Spring	Summer	Total
Total	1925300,84	1659596,00	1945796,62	2318727,78	7849421,24
Clinical mastitis	533043,22	449298,97	471504,85	607445,68	2061292,72
Subclinical mastitis	371782,58	319943,34	350393,47	401798,81	1443918,20
Healthy	1020475,04	890353,69	1123898,29	1309483,30	4344210,32

Figure 1. Economic value of cephapirin at dry-off by season (Holstein breed)

For the Simmental breed, the results presented in Figure 2 indicate a total estimated economic value of €9,781,000.99, which is approximately 25% higher than that of Holstein cows. The highest seasonal value was determined in spring (€2,709,168.41) and the lowest in winter (€2,134,674.22). Healthy cows accounted for the dominant share of the total value, ranging from 52.6% (autumn) to 59.2% (spring), while clinical mastitis contributed about 26% and subclinical mastitis about 18% of the cost. The seasonal distribution of the economic value followed the patterns of udder health structure, with higher totals observed during the warmer part of the year.



	Autumn	Winter	Spring	Summer	Total
Total	2330366,72	2134674,22	2709168,41	2606791,64	9781000,99
Clinical mastitis	647263,12	562702,10	619518,53	678351,35	2507835,10
Subclinical mastitis	457109,32	416985,59	486793,73	451698,23	1812586,86
Healthy	1225994,29	1154986,52	1602856,15	1476742,07	5460579,04

Figure 2. Economic value of cephapirin at dry-off by season (Simmental breed)

A comparison of the two breeds shows that in both populations, the highest simulated antibiotic costs were estimated in spring and summer, reflecting seasonal differences in udder health status. Holstein cows had a lower total economic value due to the smaller population size, but exhibited seasonal trends similar to those observed in Simmental cows. The obtained results confirm that seasonal fluctuations in udder health status significantly influence the economic outcome of dry cow therapy, further emphasizing the importance of rational and selective antibiotic use during the warmer months.

The estimated seasonal environmental emission of cephapirin (Cefa-Safe) and the corresponding risk quotients (RQ) are presented in Table 3. In the Holstein breed, the total emission was 221,422.32 g, with the highest seasonal value estimated for the summer period (65,408.40 g) and the lowest for winter (46,815.12 g). The predicted environmental concentrations (PEC) ranged from 46.82 μg/L in winter to 65.41 μg/L in summer, with a constant PNEC = 0.05 μg/L, resulting in risk quotients (RQ) between 936.30 and 1,308.17.

Table 3. Estimated environmental emission of cephapirin (Cefa-Safe) and associated risk quotient (RQ) by season in Holstein cows

Season	Emission (g)	PEC (μg/L)	PNEC (μg/L)	RQ (PEC/PNEC)
Autumn	54310.32	54.31	0.05	1086.21
Winter	46815.12	46.82	0.05	936.30
Spring	54888.48	54.89	0.05	1097.77
Summer	65408.40	65.41	0.05	1308.17
Total	**221422.32**	**221.42**	**0.05**	**4428.45**

Emission, PEC and RQ were calculated as described in Materials and Methods..

For the Simmental breed, the results presented in Table 4 indicate a total emission of 275,909.76 g, which is approximately 25% higher than in Holstein cows. The highest emission was recorded in spring (76,422.24 g) and the lowest in winter (60,216.48 g). The predicted environmental concentrations (PEC) ranged from 60.22 μg/L to 76.42 μg/L, while the calculated risk quotients (RQ) varied between 1,204.33 and 1,528.44.

In both breeds, the highest emissions and RQ values were observed during the warmer seasons (spring and summer), corresponding to the greater total amount of simulated antibiotic use. RQ values exceeding 1000 across all seasons indicate a significant potential for environmental risk under the BDCT model.

Table 4. Estimated environmental emission of cephapirin (Cefa-Safe) and associated risk quotient (RQ) by season in Simmental cows

Season	Emission (g)	PEC (μg/L)	PNEC (μg/L)	RQ (PEC/PNEC)
Autumn	65736.72	65.74	0.05	1314.73
Winter	60216.48	60.22	0.05	1204.33
Spring	76422.24	76.42	0.05	1528.44
Summer	73534.32	73.53	0.05	1470.69
Total	**275909.76**	**275.91**	**0.05**	**5518.20**

Emission, PEC and RQ were calculated as described in Materials and Methods.

DISCUSSION

The obtained results confirm pronounced seasonal differences in simulated cephapirin usage in dry cow therapy, with higher values during the warmer part of the year. Such a pattern is consistent with findings on the impact of heat stress and microclimatic fluctuations on increased somatic cell count (SCC) and reduced udder resistance [20,23,32]. The seasonal correlation between higher temperatures and increased mastitis prevalence has been confirmed in several studies [21,40,41], indicating that physiological stress and environmental conditions jointly elevate the risk of intramammary infections. This may explain the higher simulated cephapirin use in spring and summer, particularly in the Simmental breed.

Cephapirin, a first-generation cephalosporin, exhibits high efficacy against the most important mastitis pathogens, including *Staphylococcus aureus* and *Streptococcus uberis* [42]. Stockler et al. [7] demonstrated that, following intramammary administration, cephapirin is rapidly metabolized to desacetyl-cephapirin, which retains antibacterial activity and maintains concentrations above the minimum inhibitory concentration (MIC) for an extended period. Constable and Morin [39] reported that the MIC range for cephapirin against the main mastitis pathogens is 0.12–0.5 μg/mL, while Cortinhas et al. [38] emphasized that different cephalosporin metabolites may vary in antimicrobial efficacy, which is relevant for evaluating therapeutic outcomes and resistance control. These data confirm the pharmacological potential of cephapirin in treating intramammary infections but also highlight the need for prudent indication and targeted application to prevent unnecessary use.

The economic assessment results indicate that seasonal changes in herd health structure directly influence the costs of dry cow therapy. The highest simulated costs were observed in spring and summer, whereas winter values were the lowest. Vissio et al. [17] reported that rational treatment planning, considering udder health structure and mastitis prevalence, can reduce costs without negatively affecting udder health.

Similarly, Kovačević et al. [43] reported substantial cost differences among mastitis treatment protocols, emphasizing the potential for optimizing antibiotic expenditure.

Navaei et al. [18] confirmed that selective dry cow therapy (SDCT) significantly reduces both antibiotic usage and treatment costs compared to a non-selective (blanket) approach. In addition to the economic benefits, Kovačević et al. [44] reported that higher antibiotic use intensity is directly associated with the emergence of antimicrobial resistance in dairy herds. These complementary findings support the interpretation that seasonal surveillance of infection prevalence can serve as a valuable tool in strategic antibiotic management and the implementation of selective treatment protocols.

Estimated risk quotient (RQ) values exceeding 1,000 across all seasons indicate a potentially high environmental risk associated with cephapirin usage. According to Berendsen et al. [27] and Thiele-Bruhn [28], a significant proportion of administered antibiotics can be excreted in active form via urine and feces, reaching soil and aquatic ecosystems. Robles-Jimenez et al. [45] demonstrated that the presence of antibiotics in soil and manure can alter the microbial community composition and promote the spread of antimicrobial resistance genes. Bengtsson-Palme and Larsson [10] and Tell et al. [31] proposed a PNEC value of 0.05 μg/L as a threshold below which the risk of resistance selection is minimal. In the present model, all estimated environmental concentrations (PEC) exceeded this threshold, indicating a potential environmental hazard in the event of widespread antibiotic use during the dry cow period. Ribeiro et al. [29] and Ribeiro et al. [30] further reported that cephalosporin degradation products, including cephapirin metabolites, may exhibit greater persistence and higher ecotoxicity than the parent compound, particularly in aquatic environments. Similar findings were reported by Cvetnić et al. [11], who highlighted the persistence and environmental dissemination of mastitis pathogens and their adaptation to antimicrobial residues.

Seasonal variability thus affects all three dimensions — biological, economic, and environmental. Gantner et al. [20], and Weber et al. [32] demonstrated that heat stress increases somatic cell count (SCC) and compromises udder health, whereas Vissio et al. [17] and Navaei et al. [18] confirmed that rational treatment planning can significantly reduce antibiotic consumption. Studies by Weber et al. [8], Rowe et al. [9], McCubbin et al. [13], and Pavesi et al. [14] have shown that a selective dry cow therapy (SDCT) approach enables a reduction in antibiotic use without impairing udder health, thereby simultaneously mitigating economic and environmental risks. In addition, alternative non-antibiotic approaches such as phytotherapy, nanotechnology, and bacteriophage applications have been increasingly explored as sustainable adjuncts to selective therapy [46,47].

The results of this analysis, based on simulation models derived from real HAPIH data, confirm the importance of seasonal assessments in developing sustainable udder health management strategies and in reducing overall potential antibiotic usage in dairy production systems.

CONCLUSION

The results of the analysis indicate a clear seasonal relationship between udder health status, simulated cephapirin usage, and the estimated environmental risk. During spring and summer, when heat stress and microclimatic conditions favor a higher mastitis prevalence, the total amount of antibiotic used and the corresponding economic and ecotoxicological indicators increase. In the colder months, characterized by a lower incidence of intramammary infections, the overall need for antimicrobial therapy is reduced, confirming the importance of adopting a seasonally adjusted approach in planning dry cow therapy.

The obtained risk quotients (RQ) exceeding 1,000 in all seasons indicate a high potential for environmental risk in the event of widespread cephapirin use under blanket dry cow therapy (BDCT). These results therefore support the transition to selective strategies, which have been confirmed in the literature as equally effective in maintaining udder health, while significantly reducing antibiotic consumption and minimizing environmental impact.

The seasonal assessment thus represents an important step towards a more precise, sustainable, and scientifically grounded management of dry cow therapy in dairy production systems. The proposed modelling approach contributes to evidence-based decision-making and highlights the importance of seasonally adapted and selective antimicrobial strategies for reducing potential antibiotic use in sustainable dairy production systems.

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Ethical Statement

The study was based exclusively on the analysis of official milk recording data from HAPIH and did not involve any experimental work or handling of live animals. Therefore, no ethical approval was required. All procedures were performed in accordance with national data protection and animal welfare regulations.

Authors' contributions

BLJ designed and wrote the manuscript draft. MS and NM helped to draft the manuscript, ZS and MG participated in the analysis of the results. VG performed the statistical analysis, review and editing of the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests

The corresponding author declares that none of the authors has any competing interests.

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REFERENCES

1. Seegers H, Fourichon C, Beaudeau F: Production effects related to mastitis and mastitis economics in dairy cattle herds. Vet Res 2003, 34(5):475–491.
2. Green MJ, Green LE, Medley GF, Schukken YH, Bradley AJ: Influence of dry period bacterial intramammary infection on clinical mastitis in dairy cows. J Dairy Sci 2002, 85(10):2589–2599.
3. Benić M, Maćešić N, Cvetnić L, Habrun B, Cvetnić Ž, Turk R, Đuričić D, Lojkić M, Dobranić V, Valpotić H, Grizelj J, Gračner D, Grbavac J, Samardžija M: Bovine mastitis – a persistent and evolving problem requiring novel approaches for its control. Vet Arhiv 2018, 88(4):535–557.
4. Halasa T, Huijps K, Østerås O, Hogeveen H: Economic effects of bovine mastitis and mastitis management – a review. Vet Q 2007, 29(1):18–31.
5. Gantner V, Jožef I, Samardžija M, Steiner Z, Gantner R, Solić D, Potočnik K: The variability in the prevalence of subclinical and clinical mastitis and its impact on milk yield of Holstein and Simmental cows as a result of parity. Vet Arhiv 2024, 94(4):269–284.
6. Cvetnić L, Samardžija M, Duvnjak S, Habrun B, Cvetnić M, Jaki Tkalec V, Đuričić D, Benić M: Multi locus sequence typing and spa typing of Staphylococcus aureus isolated from the milk of cows with subclinical mastitis in Croatia. Microorganisms 2021, 9(4):725.
7. Stockler RM, Morin DE, Lantz RK, Constable PD: Effect of milking frequency and dosing interval on the pharmacokinetics of cephapirin after intramammary infusion in lactating dairy cows. J Dairy Sci 2009, 92(9):4262–4275.
8. Weber J, Borchardt S, Seidel J, Schreiter R, Wehrle F, Donat K, Freick M: Effects of selective dry cow treatment on intramammary infection risk after calving, cure risk during the dry period, and antibiotic use at drying-off – a systematic review and meta-analysis of current literature (2000–2021). Animals 2021, 11(12):3403.

9. Rowe SM, Godden SM, Nydam DV, Gorden PJ, Lago A, Vasquez AK, Royster E, Timmerman J, Thomas MJ: Randomized controlled trial investigating the effect of two selective dry-cow therapy protocols on udder health and performance in the subsequent lactation. J Dairy Sci 2020, 103(7):6493–6503.
10. Bengtsson-Palme J, Larsson DGJ: Concentrations of antibiotics predicted to select for resistant bacteria – proposed limits for environmental regulation. Environ Int 2016, 86:140–149.
11. Cvetnić L, Benić M, Cvetnić Ž, Duvnjak S, Reil I, Zdelar-Tuk M, Cvetnić M, Katalinić-Janković V, Habrun B, Kompes G, Špičić S: Bovine mastitis caused by rapid-growth environmental mycobacteria. Vet Stn (Online) 2022, 53(5):493–501.
12. Vanhoudt A, Hees-Huijps K van, Knegsel ATM van, Sampimon OC, Vernooij JCM, Nielen M, van Werven T: Effects of reduced intramammary antimicrobial use during the dry period on udder health in Dutch dairy herds. J Dairy Sci 2018, 101(4):3248–3260.
13. McCubbin KD, De Jong E, Brummelhuis CM, Bodaneze J, Biesheuvel M, Kelton DF, Uyama T, Dufour S, Sanchez J, Rizzo D, Léger D, Barkema HW: Antimicrobial and teat sealant use and selection criteria at dry-off on Canadian dairy farms. J Dairy Sci 2023, 106(10):7104–7116.
14. Pavesi FL, Pollera C, Sala G, Cremonesi P, Monistero V, Biscarini F, Bronzo V: Effect of the selective dry cow therapy on udder health and milk microbiota. Antibiotics 2023, 12:1259.
15. Müller S, Nitz J, Tellen A, Klocke D, Krömker V: Effect of antibiotic compared to non-antibiotic dry cow treatment on the bacteriological cure of intramammary infections during the dry period – a retrospective cross-sectional study. Antibiotics 2023, 12(3):429.
16. Lipkens Z, Piepers S, Vliegher SD: Impact of selective dry cow therapy on antimicrobial consumption, udder health, milk yield and culling hazard in commercial dairy herds. Antibiotics 2023, 12:901.
17. Vissio C, Richardet M, Issaly LC, Larriestra AJ: Decision making on dry cow therapy – economic evaluation using field data under Argentinian production conditions. Ciênc Agrotec 2023, 47:e016322.
18. Navaei H, Vodjgani M, Khoramian B, Akbarinejad V, Gharagozloo F, Garoussi MT, Momeni A: Evaluation of a new method of selective dry cow treatment using microbiological culture and antibiogram results. BMC Vet Res 2025, 21(1).
19. Gantner V, Steiner Z, Jožef I, Gantner R, Solić D, Potočnik K: The variability in cow's recovery potential regarding the milk recording season. Mljekarstvo 2024, 74(2):156–165.
20. Gantner V, Šinka D, Popović V, Ćosić M, Sudarić T, Gantner R: The variability of microclimate parameters in dairy cattle farm facility. In: Sustainable agriculture and rural development – Thematic proceedings of the III international scientific conference, December 2022, Belgrade. Belgrade: Institute of Agricultural Economics; 2023, 77–86.
21. Yang L, Yang Q, Yi M, Pang ZH, Xiong BH: Effects of seasonal change and parity on raw milk composition and related indices in Chinese Holstein cows in northern China. J Dairy Sci 2013, 96(11):6863–6869.
22. Heck JML, Van Valenberg HJF, Dijkstra J, Van Hooijdonk ACM: Seasonal variation in the Dutch bovine raw milk composition. J Dairy Sci 2009, 92(10):4745–4755.
23. Cerutti RD, Scaglione MC, Arfuso F, Rizzo M, Piccione G: Seasonal variations of some hematochemical parameters in Holstein bovine under the same livestock conditions. Vet Arhiv 2018, 88(3):309–321.

24. Nantapo C, Muchenje V: Winter and spring variation in daily milk yield and mineral composition of Jersey, Friesian cows and their crosses under a pasture-based dairy system. S Afr J Anim Sci 2014, 43(5):17.
25. Tomanić D, Samardžija M, Stančić I, Kladar N, Maćešić N, Kovačević Z: Mastitis challenges in Serbian dairy farming – a study on somatic cell counts and pathogen distribution. Mljekarstvo 2024, 74(3):239–248.
26. Mićić N, Stanojević D, Samolovac L, Petričević V, Stojiljković N, Gantner V, Bogdanović V: The effect of animal-related and environmental factors on daily milk production of dairy cows under heat stress conditions. Mljekarstvo 2022, 72(4):250–260.
27. Berendsen BJA, Wegh RS, Memelink J, Zuidema T, Stolker LAM: The analysis of animal faeces as a tool to monitor antibiotic usage. Talanta 2015, 132:258–268.
28. Thiele-Bruhn S: Pharmaceutical antibiotic compounds in soils – a review. Z Pflanzenernähr Bodenk 2003, 166(2):145–167.
29. Ribeiro AR, Sures B, Schmidt TC: Ecotoxicity of the two veterinary antibiotics ceftiofur and cefapirin before and after photo-transformation. Sci Total Environ 2018, 619–620:866–873.
30. Ribeiro AR, Sures B, Schmidt TC: Cephalosporin antibiotics in the aquatic environment – a critical review of occurrence, fate, ecotoxicity and removal technologies. Environ Pollut 2018, 241:1153–1166.
31. Tell J, Caldwell DJ, Häner A, Hellstern J, Hoeger B, Journel R, Mastrocco F, Ryan JJ, Snape J, Straub JO, Vestel J: Science-based targets for antibiotics in receiving waters from pharmaceutical manufacturing operations. Integr Environ Assess Manag 2019, 15(3):312–319.
32. Weber CT, Schneider CLC, Busanello M, Calgaro JLB, Fioresi J, Gehrke CR, Conceição J, Haygert-Velho IMP: Season effects on the composition of milk produced by a Holstein herd managed under semi-confinement followed by compost bedded dairy barn management. Semin Ciênc Agrár 2020, 42(5):1667–1678.
33. HAPIH: Godišnje izvješće za 2024. godinu – Govedarstvo. Hrvatska agencija za poljoprivredu i hranu. Centar za stočarstvo. 2025
34. Ljubojević B, Gantner R, Samardžija M, Potočnik K, Maćešić N, Solić D, Gantner V: Cephapirin usage at dry-off in Holstein and Simmental cows in relation to udder health, parity, and production level. Vet. Arhiv 96, 212-225, 2026.
35. Hillerton, J E: Redefining mastitis based on somatic cell count. IDF Bulletin 1999, 345:4-6.
36. SAS Institute Inc.: SAS/STAT® 9.4 User's Guide. Cary, NC: SAS Institute Inc. 2019
37. Medical Intertrade: Offer for antibiotics (Mastidry, Cefa-Safe, Orbeseal, Keraseal). Zagreb: Internal business document; June 12, 2025.
38. Cortinhas CS, Oliveira L, Hulland CA, Santos MV, Ruegg PL: Minimum inhibitory concentrations of cephalosporin compounds and their active metabolites for selected mastitis pathogens. Am J Vet Res 2013, 74(5):683–690.
39. Constable PD, Morin DE: Use of antimicrobial susceptibility testing of bacterial pathogens isolated from the milk of dairy cows with clinical mastitis to predict response to treatment with cephapirin and oxytetracycline. J Am Vet Med Assoc 2002, 221(1):103–108.
40. Tomazi T, Ferreira GC, Orsi AM, Gonçalves JL, Ospina PA, Nydam DV, Moroni P, Dos Santos MV: Association of herd-level risk factors and incidence rate of clinical mastitis in 20 Brazilian dairy herds. Prev Vet Med 2018, 161:9–18.

41. Nóbrega DB, Langoni H: Breed and season influence on milk quality parameters and mastitis occurrence. Pesq Vet Bras 2011, 31(12):1045–1052.
42. Barlow J: Mastitis therapy and antimicrobial susceptibility – a multispecies review with a focus on antibiotic treatment of mastitis in dairy cattle. J Mammary Gland Biol Neoplasia 2011, 16(4):383–407.
43. Kovačević Z, Mihajlović J, Mugoša S, Horvat O, Tomanić D, Kladar N, Samardžija M: Pharmacoeconomic analysis of the different therapeutic approaches in control of bovine mastitis – phytotherapy and antimicrobial treatment. Antibiotics 2022, 12(1):11.
44. Kovačević Z, Samardžija M, Horvat O, Tomanić D, Radinović M, Bijelić K, Vukomanović AG, Kladar N: Is there a relationship between antimicrobial use and antibiotic resistance of the most common mastitis pathogens in dairy cows? Antibiotics 2022, 12(1):3.
45. Robles-Jimenez LE, Aranda-Aguirre E, Castelan-Ortega OA, Shettino-Bermudez BS, Ortiz-Salinas R, Miranda M, Li X, Angeles-Hernandez JC, Vargas-Bello-Pérez E, Gonzalez-Ronquillo M: Worldwide traceability of antibiotic residues from livestock in wastewater and soil – a systematic review. Animals 2021, 12(1):60.
46. Mimoune N, Saidi R, Benadjel O, Khelef D, Kaidi R: Alternative treatment of bovine mastitis. Vet Stn (Online) 2021, 52(6):639–649.
47. Tomanić D, Samardžija M, Kovačević Z: Alternatives to antimicrobial treatment in bovine mastitis therapy – a review. Antibiotics 2023, 12(4):683.

SEZONSKA VARIJABILNOST SIMULIRANE PRIMENE CEFAPIRINA U PERIODU ZASUŠENJA I PRATEĆI EKOLOŠKI I EKONOMSKI EFEKTI KOD HOLŠTAJN I SIMENTALSKIH KRAVA

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Cilj ovog istraživanja bio je da se proceni potencijal za smanjenje upotrebe antibiotika tokom zasušenja mlečnih krava i povezani uticaji na životnu sredinu, simulacijom potencijalne primene cefapirina (Cefa-Safe) u zavisnosti od sezone kontrole mlečnosti i zdravstvenog statusa vimena populacije mlečnih krava u Hrvatskoj. Za analizu su korišćeni zvanični podaci kontrole mlečnosti Hrvatske agencije za poljoprivredu i hranu (HAPIH) prikupljeni u periodu od 2013. do 2022. godine. Na osnovu ovih podataka, krave su klasifikovane prema zdravstvenom statusu vimena: zdrave (<200000 ćelija/mL), subklinički mastitis (200000–400000 ćelija/mL) i klinički mastitis (>400000 ćelija/mL).

Na osnovu ovih udela simulirani su ukupna količina cefapirina, njegova ekonomska vrednost i potencijalna emisija u životnu sredinu u okviru standardnog pristupa neselektivne terapije u zasušenju (BDCT). Analiza je sprovedena po sezonama (proleće, leto, jesen, zima). Najveća procenjena upotreba antibiotika i emisije zabeležene su to-

kom leta kod holštajn krava i tokom proleća kod simentalskih krava, dok su najniže vrednosti utvrđene zimi kod obe rase.

Izračunati koeficijenti rizika (RQ = PEC/PNEC) premašili su graničnu vrednost RQ > 1 u svim sezonama, što ukazuje na postojan ekološki rizik. Ekonomske procene pratile su isti obrazac, sa najvećim troškovima u toplijem delu godine.

Rezultati potvrđuju da sezonske varijacije utiču na zdravstveni status vimena, a time i na količinu antibiotika koja je potencijalno potrebna tokom perioda zasušenja. Predloženi model omogućava kvantitativnu procenu potencijala za smanjenje upotrebe antibiotika i pruža naučnu osnovu za prelazak sa standardne na selektivnu terapiju u zasušenju (SDCT) u okviru održive proizvodnje mleka.