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Effects of intramammary infection and dry-off treatment on the immune-metabolic profile of Alpine dairy goats

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ABSTRACT

Sixty Alpine dairy goats were classified as healthy (HEAL, n = 30) or infected (INFE, n = 30) based on bacteriological culture of udder-half samples collected 7 d before dry-off. At −61 d from kidding (DFK), goats were dried off and randomly allocated to 2 homogeneous groups either receiving no treatment (15 HEAL, 15 INFE) or being treated intramammarily with 250 mg of cefazolin per half-udder (15 HEAL, 15 INFE). Milk yield, composition, and SCC were monitored at −82, 17, 45, and 80 DFK, and blood samples were collected at −66, −56, −7, and 8 DFK to assess plasma analytes. Antibiotic administration at dry-off did not affect productive performances in the new lactation or plasma analyte trends. Regardless of udder health status, lactose decreased in late lactation despite stable yield, likely due to increased SCC and mammary permeability during involution. The INFE goats had higher SCC before dry-off and higher SCS at 45 DFK. Following dry-off, plasma urea decreased across all groups, reflecting dietary changes aimed at easing milk cessation. Glucose and nonesterified fatty acids (NEFA) remained stable during the dry period. The INFE goats showed elevated plasma cholesterol at −57 DFK, suggesting transient dyslipidemia due to IMI. After kidding, all goats showed peaks in NEFA, NEFA/albumin ratio, BHB, bilirubin, and glutamate oxaloacetate transaminase, indicating body reserve mobilization and hepatic stress. Concurrently, increases in haptoglobin and ceruloplasmin, and decreases in albumin/globulin ratio and paraoxonase, reflect an acute phase response. The INFE goats showed higher reactive oxygen metabolites and thiol concentrations between −57 and 8 DFK, and elevated ceruloplasmin at −7 and 8 DFK, indicating sustained systemic inflammation. Plasma analytes could serve as effective diagnostic tools to improve the detection of subclinical mastitis in dairy goats.

Key words: metabolic profile, peripartum goat, plasma analytes, subclinical mastitis

INTRODUCTION

The prevalence of IMI in dairy goat herds has been documented as exceeding 23% in early lactation, and it increases markedly as lactation progresses (McDougall et al., 2014). Despite this high prevalence, detecting mastitis signs remains challenging, and bacterial culture (**BC**) continues to represent the gold standard for monitoring udder health in this species (McDougall et al., 2010). The limited availability of on-farm systems for continuous monitoring of individual milk yield and composition in small ruminants, together with the low frequency of monthly recording programs, hampers the timely detection of alterations driven by subclinical mastitis during lactation (Miller and Lu, 2019). Moreover, the cyclic renewal of the mammary gland throughout the lactation cycle reduces the diagnostic

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reliability of markers commonly used in dairy cows (e.g., SCC, conductivity, enzymatic activity). Similarly, the California Mastitis Test, although widely applied in cows, is less reliable in goats because it is operator-dependent, prone to false positives due to cytoplasmic particles, and difficult to integrate into routine milking (Poutrel and Lerondelle, 1983; McDougall et al., 2001).

Most dairy goat herds dry off their does approximately 60 d before the expected kidding date, as a 2-mo dry period is considered fundamental for optimizing milk yield and composition in the subsequent lactation (Caja et al., 2006). Beyond its productive benefits, dry-off represents a key stage for udder health monitoring and IMI management. Indeed, the limited availability of drugs licensed for goats and the extended withdrawal times associated with off-label treatments complicate therapeutic interventions during lactation. Consequently, routine dry-off practices often include performing BC on individual udder halves before cessation of milking to detect IMI and guide decisions on antibiotic treatment. However, dry-off also constitutes a critical bottleneck in the productive cycle of high-yielding dairy goats. The interruption of the milking routine and dietary adjustments adopted at this stage expose animals to stressors that may predispose them to pregnancy toxemia (Zobel et al., 2015), a metabolic disease characterized by hepatic steatosis, ketogenesis, systemic inflammation, and altered redox balance driven by the rapid growth of multiple fetuses in late gestation (Mongini and Van Saun, 2023). Recent studies have further documented local immune activation in goats capable of eliciting systemic inflammatory responses (Purba et al., 2020a,b). In this context, IMI at dry-off may impair the metabolic adaptation to late gestation by triggering systemic inflammation and thereby compromising key metabolic functions. Although poorly investigated in dairy goats approaching dry-off and kidding, the interplay between immune activation and metabolic regulation—referred to as immunometabolism—has been extensively described in peripartum dairy cows (Trevisi et al., 2025), where plasma analytes have shown great potential for reflecting the magnitude of these underlying processes. To date, plasma biomarker trends in goats remain scarcely studied (Mezzetti et al., 2024), and no work has evaluated the impact of mammary gland health status on immunometabolic adaptation from dry-off to kidding.

Therefore, the aim of the present study was to investigate changes in plasma analytes in 60 Alpine dairy goats from dry-off to kidding, and to evaluate the effects of BC status and antibiotic treatment at dry-off on plasma biomarker dynamics. We hypothesized that IMI compromises metabolic adaptation by establishing systemic inflammation, as reflected in altered plasma analyte patterns, and that antibiotic treatment at dry-off may modulate these processes.

MATERIALS AND METHODS

Animal Management

This study was conducted on a commercial dairy farm ("Le Camosciate di San Tommaso," San Rocco al Porto, Lodi, Italy) in accordance with Italian legislation on animal experimentation (Legislative Decree No. 116, 27/01/1992), between November 2022 and April 2023. The farm consisted of 150 Alpine dairy goats, averaging 747 L of milk per lactation (3.8 g/100 g of fat, 3.5 g/100 g of protein, 4.6 g/100 g of lactose, 2.6 g/100 g of casein, 44.1 mg/dL urea, and 2,238,000 SCC/mL in the bulk tank). Throughout the year, goats were housed in 3 group pens with deep straw bedding based on their parity status: one pen including goats at their first and second kidding, one pen including goats at their third kidding, and one pen including multiparous goats. The breeding season occurred between September and October 2022, following the goats' natural reproductive cycle. Mating was performed through natural breeding in group pens with fertile bucks, and estrus was synchronized by the buck effect. Estrous behavior was visually monitored by the farmer, and the presumed mating date for each doe was estimated from observed mountings and the absence of return to estrus. Pregnancy diagnosis and litter size estimation were subsequently performed by a veterinary practitioner using transabdominal ultrasound between November and December 2022 (i.e., approximately 40–60 d after the end of the mating period). Milking was performed twice daily at 0500 and 1600 h in a 12-unit milking parlor (Alfa Laval Corporate AB, Lund, Sweden). The milking routine included the application of a foaming pre-dipping solution (Peroxifoam, Devidet srl., Verona, Italy), drying of the teats, and stripping of the first milk, followed by attachment of the milking cluster. Cluster removal was manually performed by the operator based on udder emptying. As a final step, postdipping was applied using an iodine-based solution (Mida San 301, Christeyns, Milano, Italy). Goats were dried off after being thoroughly milked, and dry-off date for each group of goats was scheduled to ensure an average dry period of 60 d for the group based on the expected kidding dates of individual goats. This approach, rather than drying off each doe individually, was adopted to minimize the stress associated with frequent regrouping and social hierarchy changes during late gestation. Kidding took place between February and March 2023.

Feeding Routine and Diet Composition

Goats were fed a component-based diet, consisting of ad libitum access to hays and multiple concentrate meals individually administered by the farmer using a gradu-

Table 1. Composition (% of DM unless otherwise noted) and characteristics of the diets fed during the dry and lactation periods

	Days from kidding[1]							
Item	1; 283	−81; −75	−74; −68	−67; −57	−56; −46	−45; −29	−28; −16	−15; 0
DMI,[2] kg	3.21	3.20	2.89	2.54	1.86	2.13	1.92	1.97
Diet								
Chopped hay[3]	64.8	—	—	—	—	75.6	68.2	64.6
Grass hay[3]	—	64.7	76.0	89.8	86.0	—	—	—
Ground corn	8.1	8.1	9.0	10.2	—	—	4.8	8.8
Concentrate (dry period)[4]	—	—	—	—	14.0	12.2	13.5	13.3
Concentrate (lactation period)[4]	27.1	27.2	15.0	—	—	12.2	13.5	13.3
Chemical composition								
UFL[5]/kg of DM	0.84	0.91	0.93	0.81	0.81	0.77	0.81	0.83
CP	13.5	12.2	10.3	8.0	8.8	12.1	12.3	12.2
Starch + sugar	15.3	22.5	23.2	24.1	24.5	15.1	19.1	21.9
Ether extract	2.3	2.2	1.7	1.2	1.2	1.7	1.9	1.9
NDF	48.9	50.7	54.2	58.4	56.2	51.4	48.1	46.2

[1]Period during which the diet was fed, expressed as days relative to parturition.

[2]Estimated with the INRA (2018) equation.

[3]Offered ad libitum and calculated basing on the INRA (2018) equation to fulfill DMI of the goats.

[4]Dry period concentrate was composed of 80% corn flour, 4% calcium phosphate, 3.6% molasses, 3.6% wheat bran, 3.2% trace mineral and vitamin supplement, 1.6% urea, 1.6% calcium carbonate, 1.2% calcium sulfate, and 1.2% rumen-protected methionine; (supplied as Kessent M, Kemin Industries, Des Moines, IA) on a DM basis and provided 33,200 IU of vitamin A, 6,400 IU of vitamin D_3, 976.9 IU of vitamin E, 459.6 mg of Zn, 91.7 mg of Cu, 2.64 mg of Se, 5.84 mg of I, 2.91 mg of Co, 293.4 mg of Mn, and 243 mg of Fe/kg of DM. Lactation-period concentrate was composed of 30.53% soybean hulls, 29.58% soybean meal (44% CP), 10.95% beet pulp, 8.84% corn flour, 5.26% molasses, 4.21% extruded linseed, 4.10% trace mineral and vitamin supplement, 3.16% roasted soybean, 1.05% palmitic acid, 1.05% carob meal, 1.05% sodium bicarbonate, and 0.21% Smartamine on a DM basis and provided 13,100 IU of vitamin A, 2,900 IU of vitamin D_3, 36.2 IU of vitamin E, 5.5 mg of vitamin B_1, 233.3 mg of Zn, 25.3 mg of Cu, 0.71 mg of Se, 1.76 mg of I, 1.67 mg of Co, 146.4 mg of Mn, and 387 mg of Fe/kg of DM.

[5]UFL = unité fourragère lait.

ated scoop to ensure that each animal received the correct amount according to the productive phase. During lactation, alfalfa and grass hay were chopped and mixed in a 1:1 weight-to-weight ratio using a mixer wagon and fed fresh 4 times daily. At each milking, goats received 150 g of ground corn. Following milking, each goat was offered 500 g of a concentrate formulated to meet the requirements of a 70-kg goat producing 4 kg/d of milk at 4.0% fat and 3.5% protein, consuming 3.5 kg/d of DM. Three weeks before dry-off, alfalfa hay was withdrawn to promote smoother adaptation to milking cessation, and 2 wk before dry-off the amount of concentrate was halved. During the final week of lactation, goats were fed grass hay and 300 g of ground corn only. Three days after dry-off, ground corn was replaced with an equal amount of a concentrate formulated to meet the gestation requirements of an 80-kg dry goat carrying triplets. In the final month before the expected kidding date, alfalfa hay and lactation concentrate were gradually reintroduced to meet the increasing nutritional needs of the developing fetuses, up to 75% of the amount provided at peak lactation. Diets were formulated according to the INRA 2018 Feeding System for Ruminants (INRA, 2018) and are detailed in Table 1 and Figure 1.

Experimental Design

The experimental design and procedures involving the goats were reviewed and approved by the Ethics Committee for Animal Welfare of the University of Milan, Italy (approval no. OPBA 118–2023) and were conducted in compliance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. Goats used in this experiment were originally enrolled in a previous trial (Hossain et al., 2025). As no published data are available on the metabolic profile of Alpine goats at dry-off, it was not possible to perform a sample size calculation tailored to plasma analytes before study initiation, and the sample size used in this experiment was defined to investigate the effect of antibiotic treatment on the mammary gland microbiome. Briefly, a total of 106 dairy goats in their first (n = 63) or second (n = 43) lactation were recruited based on the following criteria: (1) good general health with no signs of clinical mastitis, and (2) no systemic or intramammary antibiotic treatment or anti-inflammatory medication administered within −30 d from dry-off. At −7 d from dry-off, milk samples were collected from each udder half following the National Mastitis Council recommendations (Adkins and Middleton, 2017) and cultured for bacteriological analysis at the Laboratorio di Malattie Infettive degli Animali (MiLab, Università degli Studi di Milano, Lodi, Italy). For each positive sample, bacterial colonies were identified using MALDI-TOF MS with the MALDI Biotyper System (Bruker Daltonik GmbH, Bremen, Germany), using the direct transfer method. Based on BC results and blocked by parity, number of fetuses, length

Table 2. Parity, number of kids carried, length of the previous lactation, total milk yielded in the previous lactation, average SCC in the previous lactation, and dry-period length of goats included in 4 experimental groups (values presented as mean ± SD)

	CG[1]		TG[1]	
Item	HEAL[2]	INFE[2]	HEAL	INFE
Subjects, n	15	15	15	15
Parity, n	1.67 ± 0.49	1.27 ± 0.46	1.80 ± 0.41	1.33 ± 0.49
Kids carried, n	1.71 ± 0.83	1.67 ± 0.49	1.93 ± 0.59	1.60 ± 0.51
Previous lactation length, d	288.40 ± 27.31	286.20 ± 30.46	297.13 ± 5.84	287.93 ± 24.49
Previous lactation milk yield, kg	755.4 ± 270	690.2 ± 335	791.3 ± 109.4	692.4 ± 327
Previous lactation SCC, n (× 1,000)/mL	2,268.8 ± 1,536	1,829.3 ± 1,068	1,690.2 ± 1,000	2,711.7 ± 2026
Dry-period length, d	58.43 ± 22.45	60.00 ± 22.45	69.07 ± 17.33	57.60 ± 19.14

[1]CG = goat receiving no antibiotic treatment at milking interruption (n = 30); TG = goat treated with an intramammary suspension of cefazolin 250 mg (Cefovet A, Dopharma, Firenze, Italy) at milking interruption (n = 30).

[2]HEAL goats showed no signs of bacterial growth (pathogens or not) in any mammary-half 7 d before dry-off (CG-HEAL n = 15; TG-HEAL n = 15); INFE goats had at least 1 mammary-half affected by bacterial growth at 7 d before dry-off (CG-INFE n = 15; TG-INFE n = 15).

of the previous lactation, and total milk yield during the previous lactation, 60 of the 106 goats were selected for the study: 30 healthy goats with both udder-half samples BC-negative (**HEAL**) and 30 infected goats with at least one udder-half BC-positive for one or 2 pathogens (**INFE**). At −61 ± 19 d from real kidding (**DFK**), the 60 goats were included in a 2 × 2 factorial experimental design. Goats were blocked by udder health status based on BC results and randomly assigned to 2 dry-off routines: a control group (CG; 30 goats: 15 HEAL and 15 INFE), which received no treatment, and a treatment group (TG; 30 goats: 15 HEAL and 15 INFE), which received antibiotic therapy. Each udder half of TG goats was treated with an intramammary infusion of 250 mg of cefazolin (Cefovet A, Dopharma, Florence, Italy) at dry-off. The individual goats were assumed as experimental units of this experiment. Parity, number of fetuses, length, total milk yield, and average SCC during the previous lactation and dry period length for each of the 4 experimental groups are detailed in Table 2.

Animal Measurements, Sample Collection, and Handling Procedures

Periodic sampling and data collection were carried out on goats from −82 to 80 DFK, as described next and shown in Figure 2. The health status of each goat was monitored daily, and at each milking, the operator visually inspected the milk to detect any alterations indicative of clinical mastitis. Feed samples were collected at every feed batch change. Data on milk yield, composition, and SCC were obtained from DHI checks conducted on average at −82 ± 22, 17 ± 7, 45 ± 7, and 80 ± 9 DFK, alternating between morning and evening milkings. A colostrum sample was collected at kidding, and additional milk samples were collected at dry-off (−61 ± 19) and at 8 ± 2.0 DFK to assess BC results. Blood samples were collected at −66 ± 19 (−5 d from dry-off), −56 ± 19 (5 d after dry-off), −7 ± 2.0, and 8 ± 2.0 DFK via jugular venipuncture before morning feeding, using 10-mL evacuated heparinized tubes (BD Vacutainer, BD Diagnostics, Franklin Lakes, NJ). On each sampling day, BCS was assessed by the same trained operator following the method described by Villaquiran et al. (2004). After collection, blood samples were centrifuged to determine packed cell volume (**PCV**), and plasma was stored at −20°C for subsequent metabolic profile analysis.

Analytical Procedures

Bacterial Culture and Microbiological Analysis. The milk samples were cultured for bacteriological examination following the National Mastitis Council recommendations (Adkins and Middleton, 2017). Briefly, 10 µL of each milk sample was inoculated on blood agar base plates (Oxoid Ltd., Basingstoke, UK) with 5% defibrinated sheep blood. The inoculated agar plates were cultured aerobically at 37°C for 24 to 48 h, after which they were read after 24 and 48 h. Bacterial colonies were tentatively identified based on their cultural characteristics and hemolysis patterns. Samples showing growth of ≥5 similar colonies were considered positive for bacterial infection (other than *Staphylococcus aureus*), whereas samples showing growth of ≥1 similar hemolytic colony of *S. aureus* were considered positive for this pathogen. Any milk sample showing >2 different types of colonies (pathogens) was classified as contaminated (Abrahmsén et al., 2014; Song et al., 2020; Hossain et al., 2025). For further MALDI-TOF MS analysis, a small portion of a pure isolated colony was spread in the well of a target plate (MSP96 polished-steel target, Bruker Daltonik, Bremen, Germany) via a sterile toothpick and left for a few minutes to air dry. The samples were dried well, and the colonies were overlaid with 1 µL of a solution of α-cyano-

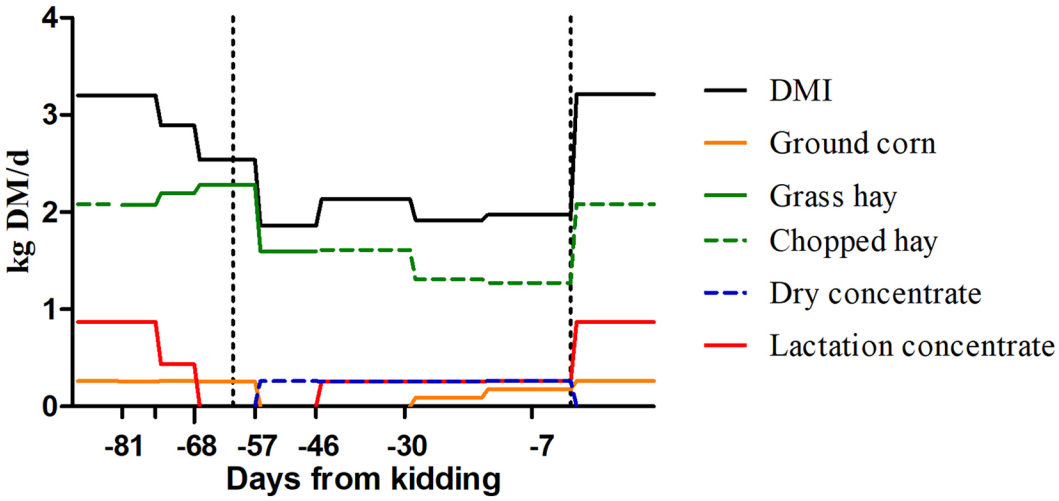

Figure 1. Theoretical rations formulated for Alpine dairy goats approaching dry-off and kidding. Hays were offered ad libitum, whereas DMI and hay consumption were estimated according to INRA (2018) equations.

4-hydroxycinnamic acid dissolved in 50% acetonitrile and 2.5% trifluoroacetic acid (Bruker Daltonik GmbH, Bremen, Germany) and allowed to dry. Then, the target plate was placed on a MALDI Biotyper Microflex LT/SH MALDI-TOF mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany), and analysis was performed according to the user manual to obtain the spectrum for each sample. The generated spectrum was further analyzed via MALDI Biotyper 2.0 software (Bruker Daltonik GmbH, Bremen, Germany), and a list of picks was produced, which was matched with the MALDI Biotyper Compass Library to identify the genus, species, and subspecies of bacteria. The identification was performed by calculating the log score with a maximum value of 3. A calculated log value ≥2.0 is considered reliable identification of a species, whereas a log score between 1.7 and 2.0 is considered a presumptive identification of a species but is reliable for genus identification. The samples with log scores <1.7 were considered unidentified (Nonnemann et al., 2013; Schulthess et al., 2013; Hossain et al., 2025; Ulloa et al., 2025). Regarding NAS identification, we considered a log value of ≥1.7 for species identification following previously published studies on MALDI-TOF MS for staphylococcal identification (Cameron et al., 2017; Wanecka et al., 2019; Jeffrey et al., 2025).

Plasma Metabolic Profile. Frozen plasma samples used to perform the analytical procedures were thawed within 3 mo from collection and processed as described here. A clinical autoanalyzer (ILAB Taurus, Instrumentation Laboratory, Bedford, MA) was used to determine the plasma concentration of glucose, nonesterified fatty acids (**NEFA**), triglycerides, BHB, urea, creatinine, calcium, phosphorus, glutamate oxaloacetate transaminase (**GOT**), gamma glutamyl transferase (**GGT**), alkaline phosphatase, total protein, haptoglobin, ceruloplasmin, albumin, total bilirubin, and cholesterol in accordance with Calamari et al. (2016). The plasma reactive oxygen metabolites (**ROMt**) and ferric ion reducing antioxidant power (**FRAP**) were determined according to Jacometo et al. (2015). The plasma concentration of paraoxonase was determined according to Bionaz et al. (2007), those of thiol according to Minuti et al. (2014), and those of myeloperoxidase according to Bradley et al. (1982). The analytical methods used for plasma biomarker assessment in this study are enzymatic–colorimetric methods, which do not rely on species-specific antibodies and have been widely applied across different animal species. Although species-specific validation in goats is limited, these assays are broadly used in caprine research (Mezzetti et al., 2024) and are considered appropriate for this species. In our laboratory, intra- and interassay CV are routinely determined on bovine plasma, as this matrix is the most frequently analyzed and available in sufficient amounts to generate robust quality control data. Assay performance in goat plasma was also verified for consistency and repeatability before use. Further details on the analytical procedures of the metabolic profile analysis are reported in Supplemental Table S1 (see Notes).

Calculations. In milk, the SCS was calculated based on SCC according to Wiggans and Shook (1987) and the ECM was calculated based on daily yield and fat, protein, and lactose contents according to Sjaunja et al. (1990). Plasma globulin content was calculated as the difference between total protein and albumin. Among

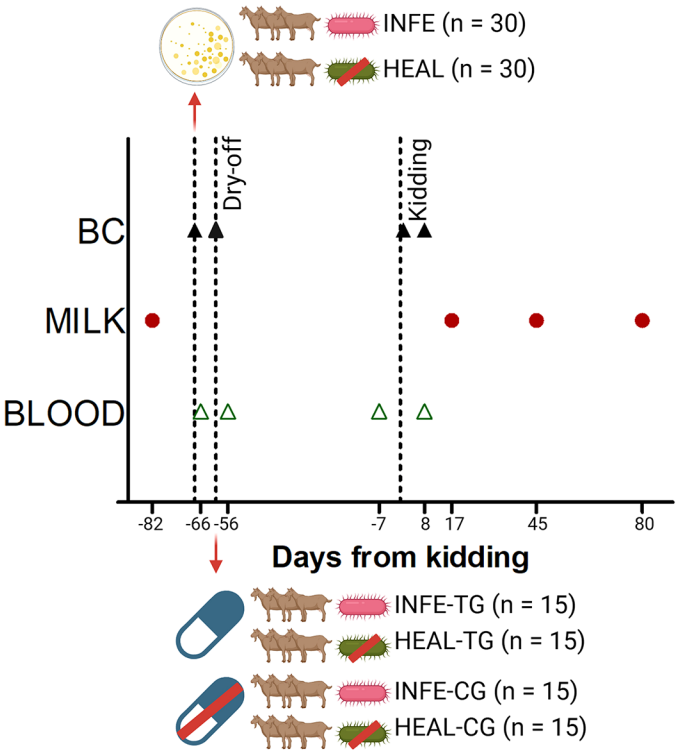

Figure 2. Scheduled time points, expressed as days from kidding, for the classification of dairy goats based on bacterial culture results (HEAL = goats showed no signs of bacterial growth in any mammary-half 7 d before dry-off; INFE = goats had at least 1 mammary-half affected by bacterial growth at 7 d before dry-off) and for the follow-up of the bacteriological status of the mammary glands (BC; black solid triangles); milk yield, butterfat, protein, lactose, and SCC recordings by DHI checks (MILK; red solid circles); and body condition scoring and blood sample collection for plasma metabolic profile determination (BLOOD; green open triangles). Dry-off is the day when milk removal was halted and goats were either treated with a intramammary cefazolin suspension (TG) or dried off without treatment (CG). Kidding is day of delivery.

plasma analyte ratios, the NEFA to albumin ratio (**NAR**) was calculated according to Gonçalves-de-Albuquerque et al. (2019) to reflect albumin saturation by circulating NEFA, the albumin to globulin ratio (**AGR**) according to Cattaneo et al. (2021) and the myeloperoxidase to paraoxonase ratio (**MPR**) according to Haraguchi et al. (2014) to reflect the systemic inflammatory status, and the ROMt to FRAP ratio (**RFR**) according to Ling et al. (2018) to reflect the redox balance.

Statistical Analysis

Data were analyzed in SAS software, version 9.4 (SAS Inst. Inc., Cary, NC) and are presented in graphs and tables as the LSM and pooled standard error for individual means of treatments over time. Data of milk yield, milk composition, SCC, BCS, and blood analytes underwent ANOVA testing using mixed models for repeated measures (Glimmix Procedure, SAS Inst. Inc.). For milk yield, milk composition, SCC, BCS, and blood analytes, the statistical model included the fixed effect of antibiotic treatment (**Treat**; CG and TG); health status of the mammary gland, as reflected by BC at dry-off (**HS**; HEAL and INFE); time; the first-order interaction effects of Treat × HS, Treat × time, and HS × time; the second-order interaction effect of Treat × HS × time; and the random effect of the individual goat. Time effect had 4 observations for MY, composition, and SCC (−82, 17, 45, and 80 DFK) and 4 observations for BCS and blood analytes (−66, −56, −7, and 8 DFK). Parity (**PAR**; 1 and 2) and number of kids carried by the goat (**KN**; 1, 2, and 3) were included in the model as a covariate. The analyses were conducted using 4 covariance structures (autoregressive, unstructured, antedependence, and spatial power) with their heterogeneous counterparts. These were ranked according to their Akaike information criterion, with the one having the lowest criterion being chosen for final statistical analysis (Littell et al., 1998). Significance was declared for $P \leq 0.05$, and differences for $P \leq 0.10$ were discussed in the context of tendencies. As the antibiotic treatment did not affect any of the explored parameters, the fixed effect of Treat, the first-order interaction effects of Treat × HS and Treat × time, and the second-order interaction effect of Treat × HS × time were removed from the final model. In the publication by Hossain et al. (2025), 5 goats (1 CG-INFE, 1 CG-HEAL, and 3 TG-INFE) were reported as excluded from the experiment. In fact, 2 goats died due to dystocia, and 3 were treated with antibiotics for retained placenta. As these events occurred after the third blood sampling, data collected before parturition were retained in the present analysis. Therefore, blood samples, BCS measurements, and milk DHI records included 60 goats for prepartum time points and 55 goats for postpartum measurements. To provide a clear and nonredundant representation of the model outcomes, LSM of the main effect of HS are presented in tables, whereas temporal dynamics and HS × time interactions are illustrated in figures.

RESULTS

Bacteriological Culture and Cure Rates

Results on the BC of the goats included in this study have been reported elsewhere (Hossain et al., 2025) and are presented in Table 3. Briefly, in CG-INFE goats, *Staphylococcus caprae* was the dominant bacterial species detected at dry-off (29%), *Staphylococcus equorum* in colostrum (33%), and *Serratia marcescens* at 8 DFK (11%). In TG-INFE goats, *S. equorum* was the dominant bacteria detected at dry-off (15%) and in colostrum (22%), whereas *Serratia*

Table 3. Distribution of bacteria (number of positive samples along with the corresponding relative percentages within parentheses) in milk samples collected at dry-off (−61 DFK), at kidding (0 DFK), and at 8 DFK from Alpine dairy goats classified as healthy (HEAL) or infected (INFE) based on milk samples collected 7 d before dry-off and either dried off without any treatment (CG) or receiving 250 mg of cefazolin intramammarily (TG)[1]

		Time, DFK							
		−61		0		8		Total	
Bacteria[2]	Treatment	INFE	HEAL	INFE	HEAL	INFE	HEAL	INFE	HEAL
Staphylococcus caprae	CG	8 (57.1)	—	4 (28.6)	3 (21.4)	6 (42.9)	—	18 (42.9)	3 (7.1)
	TG	1 (8.3)	—	1 (8.3)	1 (6.7)	1 (8.3)	—	3 (8.3)	1 (2.2)
Staphylococcus equorum	CG	4 (28.6)	—	2 (14.3)	5 (35.7)	—	—	6 (14.3)	5 (11.9)
	TG	4 (33.3)	—	4 (33.3)	1 (6.7)	—	—	8 (22.2)	1 (2.2)
Staphylococcus aureus	CG	—	—	—	—	—	—	—	—
	TG	3 (25)	—	—	—	1 (8.3)	—	4 (11.1)	—
Serratia marcescens	CG	2 (14.3)	—	—	—	2 (14.3)	1 (7.1)	4 (9.5)	1 (2.4)
	TG	3 (25)	—	3 (25)	—	2 (16.7)	—	8 (22.2)	—
Other NAS	CG	—	—	1 (7.1)	1 (7.1)	1 (7.1)	—	2 (4.8)	1 (2.4)
	TG	—	—	—	7 (46.7)	—	—	—	7 (15.6)
Others	CG	—	—	1 (7.1)	—	—	1 (7.1)	1 (2.4)	1 (2.4)
	TG	1 (8.3)	—	—	1 (6.7)	—	—	1 (2.8)	1 (2.2)
No growth	CG	—	14 (100)	5 (35.7)	5 (35.7)	3 (21.4)	12 (85.7)	8 (19)	31 (73.8)
	TG	—	15	4 (33.3)	5 (33.3)	8 (66.7)	15 (100)	12 (33.3)	35 (77.8)
Contamination	CG	—	—	1 (7.1)	—	2 (14.3)	—	3 (7.1)	—
	TG	—	—	—	—	—	—	—	—

[1]Table adapted from Hossain et al. (2025) and considering 55 healthy goats only.

[2]Other NAS includes *Staphylococcus warneri* and *Staphylococcus succinus*; other bacteria ("others") includes *Escherichia coli*, *Corynebacterium stationis*, and *Lactococcus lactis*.

marcescens was the prevalent species at 8 DFK (7%). As compared with CG-INFE, TG-INFE had a lower proportion of infected mammary glands at 8 DFK ($P = 0.02$).

Milk Yield, Composition, and Somatic Cell Count

Compared with HEAL, INFE goats had a tendency toward higher SCC at −82 DFK ($P < 0.10$) and had higher SCS at 45 DFK ($P < 0.05$; Figure 3). No effect of HS was detected on milk yield and quality parameters, whereas a significant effect of time was observed for all of them (Table 4). Milk yield and ECM were lower at −82 and 17 than at 45 and 80 DFK. The concentration of fat was higher at −82 and 17 than 80 DFK. The concentration of milk protein and casein was the highest at −82 DFK, decreased at 17 DFK and was the lowest from 45 to 80 DFK. The concentration of lactose was the lowest at −82 DFK, peaked at 17 DFK, and decreased as lactation progressed. The concentration of urea was steady from −82 to 45 DFK and peaked at 80 DFK. The SCC were highest at −82 DFK and maintained steady concentration thereafter, whereas SCS were highest at −82 DFK, dropped at 17 DFK, and rose again at 45 and 80 DFK.

Body Condition Score, Packed Cell Volume, and Plasma Metabolic Profile

Body Condition Score, Packed Cell Volume, Energy, Protein, and Mineral Metabolism Biomarkers. No effect of HS was detected on BCS, PCV or on any of the plasma analytes reflecting energy, protein, and mineral metabolism, but except for the concentration of creatinine and Ca (Supplemental Figure S1, see Notes), all the parameters were affected by time (Table 5 and Figure 4). The BCS was lowest at −66 DFK, rose during the dry period and declined again at 8 DFK, while PCV maintained steady concentration across the study and dropped at −7 DFK. The concentration of glucose and those of NEFA remained steady until 8 DFK, when the concentration of glucose had a drop and those of NEFA had a peak. The concentration of triglycerides rose from −66 DFK throughout the dry period and had a drop at 8 DFK. The concentration of BHB had a drop after dry-off, increased thereafter and peaked at 8 DFK. The concentration of urea and those of P dropped after dry-off and rose again as parturition approached.

Liver Function, Liver Damage, and Inflammation Biomarkers. Compared with HEAL, INFE goats had higher cholesterol at −57 DFK ($P < 0.01$), higher ceruloplasmin at −7 DFK ($P < 0.05$), and a tendency toward higher ceruloplasmin at 8 DFK ($P < 0.1$; Figure 6). No effect of HS was detected on the other plasma analytes, whereas a significant effect of time was observed for all of them. Most of the analytes maintained steady concentration throughout most of the study period. The concentration of bilirubin, GOT, haptoglobin, and ceruloplasmin peaked at 8 DFK. Those of GGT, total protein, globulin, and albumin dropped at −7 DFK and those of alkaline

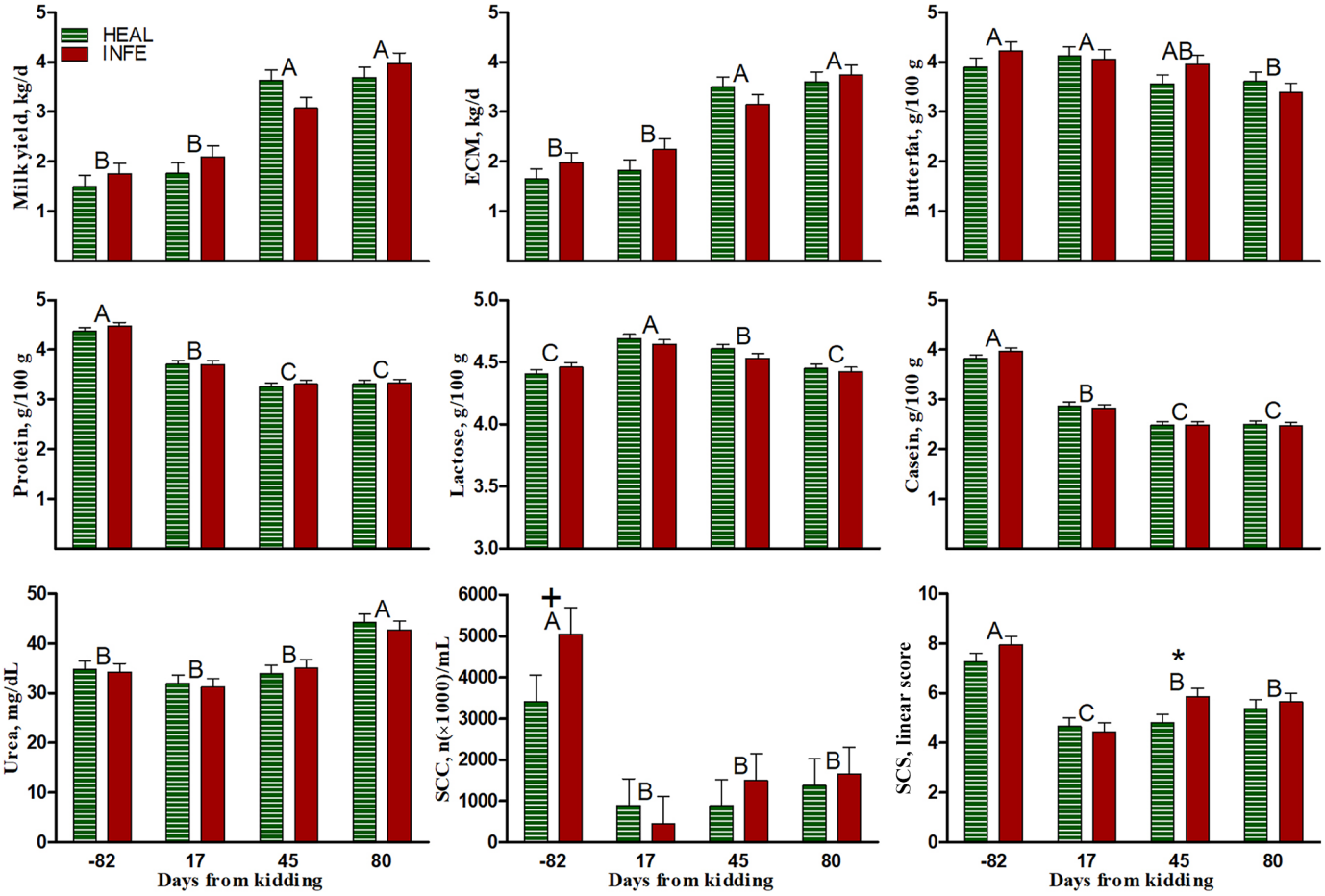

Figure 3. Pattern of milk yield, ECM, milk butterfat, protein, lactose, casein, urea, SCC, and SCS in Alpine dairy goats classified as healthy (HEAL) or infected (INFE) based on milk samples collected 7 d before dry-off. Different letters (A–C) indicate time points differing from each other ($P < 0.05$) and symbols reflect differences between HEAL and INFE group when the health status × time interaction is significant (HS × time; *$P < 0.05$; +$P < 0.1$).

phosphatase at 8 DFK. The concentration of myeloperoxidase peaked at −57 DFK and decreased gradually thereafter. The concentration of cholesterol decreased as parturition approached, whereas those of paraoxonase decreased after dry-off, remained steady during the dry period, and dropped at 8 DFK (Figures 5 and 6).

Redox Balance Biomarkers and Plasma Analyte Ratios. Compared with HEAL, INFE goats had a tendency toward higher ROMt and thiol at −57 DFK ($P = 0.10$), higher ROMt and thiol at −7 DFK ($P < 0.05$ and 0.01, respectively), and higher ROMt at 8 DFK ($P < 0.05$; Figure 8). No effect of HS was detected on the other analytes, whereas a significant effect of time was observed for all of them. The concentration of ROMt remained steady throughout the study, those of FRAP peaked at −57 DFK, and those of thiol dropped after dry-off and peaked at −7 DFK. The RFR dropped at −57 and NAR peaked at 8 DFK, remaining steady throughout the rest of study. The MPR rose after dry-off and remained steady thereafter (Figures 7 and 8).

DISCUSSION

Plasma analyte trends of dairy goats approaching dry-off and kidding are largely unknown; thus, the patterns observed in this study are noteworthy. A limitation of the design is the variability in dry-off timing relative to kidding (−61 ± 19 DFK), which resulted in different dry-period lengths and may have acted as a potential confounder. However, blood samples around dry-off were collected at the same interval from milking cessation for all goats, and those around kidding were referenced to the expected parturition date, showing limited dispersion relative to actual kidding. The most relevant metabolic adaptations efforted by dairy goats during the dry period occur during the last month of gestation (i.e., the second month of the dry period), whereas the first month is metabolically less demanding. Since all does had a dry period longer than 50 d, variability in dry-period length likely contributed only marginally to the plasma analyte trends observed, as

Table 4. Milk yield, ECM, milk quality biomarkers, and milk SCC of Alpine dairy goats classified as healthy (HEAL) or infected (INFE) based on milk samples collected 7 d before dry-off

	HS[1]			*P*-value		
Item[2]	HEAL	INFE	SEM[3]	HS	Time	HS × time
Milk yield, kg	2.64	2.72	0.113	0.66	<0.01	0.08
ECM, kg	2.64	2.78	0.118	0.44	<0.01	0.14
Butterfat, g/100 g	3.80	3.91	0.095	0.43	<0.01	0.27
Protein, g/100 g	3.66	3.70	0.041	0.52	<0.01	0.82
Lactose, g/100 g	4.54	4.51	0.026	0.59	<0.01	0.22
Casein, g/100 g	2.92	2.94	0.044	0.80	<0.01	0.53
Urea, mg/dL	36.2	35.8	0.849	0.77	<0.01	0.89
SCC, n (×1,000)/mL	1,645	2,156	502.0	0.49	<0.01	0.09
SCS, linear score	5.54	5.97	0.233	0.21	<0.01	0.06

[1]HEAL goats showed no signs of bacterial growth (pathogens or not) in any mammary-half 7 d before dry-off (n = 30); INFE goats had at least 1 mammary-half affected by bacterial growth at 7 d before dry-off (n = 30). Values are expressed as the LSM (±SEM) for the main effect of health status across all sampling points in the experiment, as estimated by the repeated measures model.

[2]ECM = [milk yield (kg/d) × (0.383 × butterfat (g/100 g) + 0.242 × protein (g/100 g) + 0.1571 × lactose (g/100 g) + 0.207)/3.14]; SCS = $\log_2$(SCC/100) + 3.

[3]SEM = largest standard error of the mean for the fixed effects.

feed restriction aimed at easing milk production did not overlap to the most demanding phase of the pregnancy for any of the goats included in the study. Enforcing an exact 60-d dry period without hormonal synchronization would have required frequent regrouping by diet and milking status, a practice prone to induce social stress, metabolic disturbances, and even traumatic abortions (Zobel et al., 2019). Thus, allowing some variability was considered a more practical and safer compromise, and one that better reflects commercial farm conditions. Nonetheless, the trends reported here should be interpreted with caution, as the study was conducted within a single commercial herd. While this minimized environmental variability and ensured homogeneous management, it may also limit the generalizability of the findings to herds with different genetics, housing, nutrition, or management systems.

Plasma Analytes Did Not Indicate Mobilization of Lipid Reserves, But Rather Reflected Increased Inflammation and Activation of the Acute Phase Response in Dairy Goats Before Parturition

In our study, the reduced plasma urea concentration observed at −66 DFK, followed by a marked decrease after dry-off compared with early lactation, reflects the induction of a transient protein deficit designed to facilitate a gradual cessation of milk production. In fact, milk and plasma urea are reliable indicators of dietary protein levels (Rapetti et al., 2014). This nutritional adjustment was achieved by progressively reducing alfalfa and lactation concentrate before dry-off. Notably, the gradual increase in BCS observed from late lactation to kidding, along with the stable levels of glucose and lipid mobilization-related plasma markers such as NEFA (Dunshea et al., 1989) are in contrast with trends documented elsewhere for late-gestating goats (Castagnino et al., 2015). Underfeeding late-gestating goats has been consistently listed as a risk factor for developing pregnancy toxemia (Laporte-Broux et al., 2011; He et al., 2015) and we could speculate that the gradual increase of lactation concentrate adopted in this study as expected kidding approached (up to 75% of dosage provided at peak of lactation) effectively mitigated the risk of negative energy balance prepartum. In this context, the trends in plasma BHB observed from late lactation through to kidding are likely influenced by the amount of concentrate supplied to the goats, as ruminal absorption of butyric acid contributes, at least in part, to circulating BHB concentrations (Loncke et al., 2015).

After kidding, our goats were immediately milked twice daily. Even though we did not have available data on the individual yields in the first few days of lactation, milk yield and composition traits observed in this study by DHI checks align with previous findings for seasonal Alpine dairy goats (Garcia-Hernandez et al., 2007; Goetsch et al., 2011), even reflecting a normal productive career for the animals included in the experiment. The substantial drop in BCS and plasma glucose in early lactation, coupled with a peak in NEFA and NAR—a marker of albumin saturation by NEFA (Gonçalves-de-Albuquerque et al., 2019)—indicates that the onset of milk production was the primary driver of body reserve mobilization in our goats, consistently with previous observations (Zamuner et al., 2020a). The postpartum BHB peak likely reflects hepatic ketogenesis (Zamuner et al., 2020b) potentially due to increased NEFA availability and impaired hepatic re-esterification in early lactation, as previously reported and supported here by the reduction in plasma triglycer-

Table 5. Body condition score; packed cell volume; plasma analytes reflecting energy, protein, and mineral metabolism; liver function; inflammation; redox balance; and ratios between plasma analytes of Alpine dairy goats classified as healthy (HEAL) or infected (INFE) based on milk samples collected 7 d before dry-off

	HS[1]			*P*-value		
Item[2]	HEAL	INFE	SEM[3]	HS	Time	HS × time
BCS	3.12	3.16	0.052	0.48	<0.01	0.64
Packed cell volume, L/L	0.28	0.28	0.004	0.45	<0.01	0.16
Energy metabolism						
Glucose, mmol/L	3.50	3.50	0.054	0.92	<0.01	0.34
NEFA, mmol/L	0.40	0.40	0.029	0.96	<0.01	0.74
Triglycerides, mmol/L	0.27	0.29	0.010	0.25	<0.01	0.17
BHB, mmol/L	0.38	0.40	0.045	0.75	<0.01	0.85
Protein metabolism						
Urea, mmol/L	6.06	5.99	0.190	0.77	<0.01	0.64
Creatinine, µmol/L	67.2	67.4	1.023	0.84	0.05	0.96
Mineral metabolism						
Calcium, mmol/L	2.36	2.37	0.025	0.73	0.66	0.79
Phosphorus, mmol/L	2.06	2.15	0.101	0.43	<0.01	0.79
Liver function and liver damage						
Bilirubin, µmol/L	1.18	1.03	0.109	0.24	<0.01	0.31
Glutamate-oxalacetate transaminase, U/L	148.4	156.8	8.468	0.41	<0.01	0.87
γ-Glutamyl transferase, U/L	43.7	43.6	2.764	0.97	<0.01	0.99
Alkaline phosphatase, U/L	116.5	156.1	24.45	0.17	<0.01	0.34
Inflammation						
Protein, g/L	74.6	75.5	0.967	0.42	<0.01	0.24
Globulin, g/L	38.6	39.6	0.932	0.36	<0.01	0.31
Myeloperoxidase, U/L	477.9	485.1	11.10	0.57	0.02	0.74
Haptoglobin, g/L	0.26	0.24	0.025	0.51	<0.01	0.28
Ceruloplasmin, µmol/L	3.74	3.90	0.108	0.20	0.01	0.02
Albumin, g/L	35.9	35.9	0.413	0.88	0.01	0.29
Cholesterol, mmol/L	2.69	2.84	0.085	0.10	<0.01	0.01
Paraoxonase, U/mL	105.8	111.8	4.499	0.26	<0.01	0.19
Redox balance						
FRAP, µmol/L	99.4	100.2	1.733	0.67	<0.01	0.37
ROMt, mg H_2O_2/100 mL	15.6	16.8	0.501	0.04	0.10	0.03
Thiol group, µmol/L	252.2	268.1	6.194	0.03	0.03	0.07
Ratio						
AGR	0.94	0.93	0.024	0.57	0.01	0.38
NAR	0.01	0.01	0.001	0.80	<0.01	0.70
RFR	0.16	0.17	0.006	0.14	<0.01	0.29
MPR	4.98	4.59	0.253	0.22	<0.01	0.60

[1]HEAL goats showed no signs of bacterial growth (pathogens or not) in any mammary-half 7 d before dry-off (n = 30); INFE goats had at least 1 mammary-half affected by bacterial growth at 7 d before dry-off (n = 30). Values are expressed as the LSM (±SEM) for the main effect of health status across all sampling points in the experiment, as estimated by the repeated measures model.

[2]FRAP = ferric ion reducing antioxidant power; ROMt = total reactive oxygen metabolites; AGR = albumin to globulin ratio; NAR = NEFA to albumin ratio; RFR = ROMt to FRAP ratio; MPR = myeloperoxidase to paraoxonase ratio.

[3]SEM = largest standard error of the mean for the fixed effects.

ides (Dunshea et al., 1990; Mezzetti et al., 2024). In addition to this, the peak concentrations of haptoglobin and ceruloplasmin detected at 7 DFK, 2 of the main positive acute phase proteins (Ceciliani et al., 2012), coupled with the concurrent decline in the AGR and paraoxonase, a known negative acute phase protein (Bionaz et al., 2007), indicate the activation of an acute phase response (**APR**) in early lactating dairy goats similar to that described in cows. Concurrently, the sharp rise in circulating bilirubin and GOT possibly reflects reduced hepatic clearance capacity and potential liver damage (Rodriguez-Jimenez et al., 2018; Ghavipanje et al., 2021). These phenomena are likely linked, as the APR that affects the liver following parturition is a well-recognized trigger of impaired liver function and increased ketogenesis in early lactating dairy cows (Bertoni et al., 2008). However, differently from cows, the decrease in albumin and cholesterol (which reflects circulating lipoprotein levels) were already evident at −7 DFK in our goats. These results are consistent with those of previous studies (D'angelo et al., 2005; Mezzetti et al., 2024), suggesting that parturition may not represent the sole—or even the primary—stimulus for APR in goats, and that other physiological processes contribute to the onset of this response.

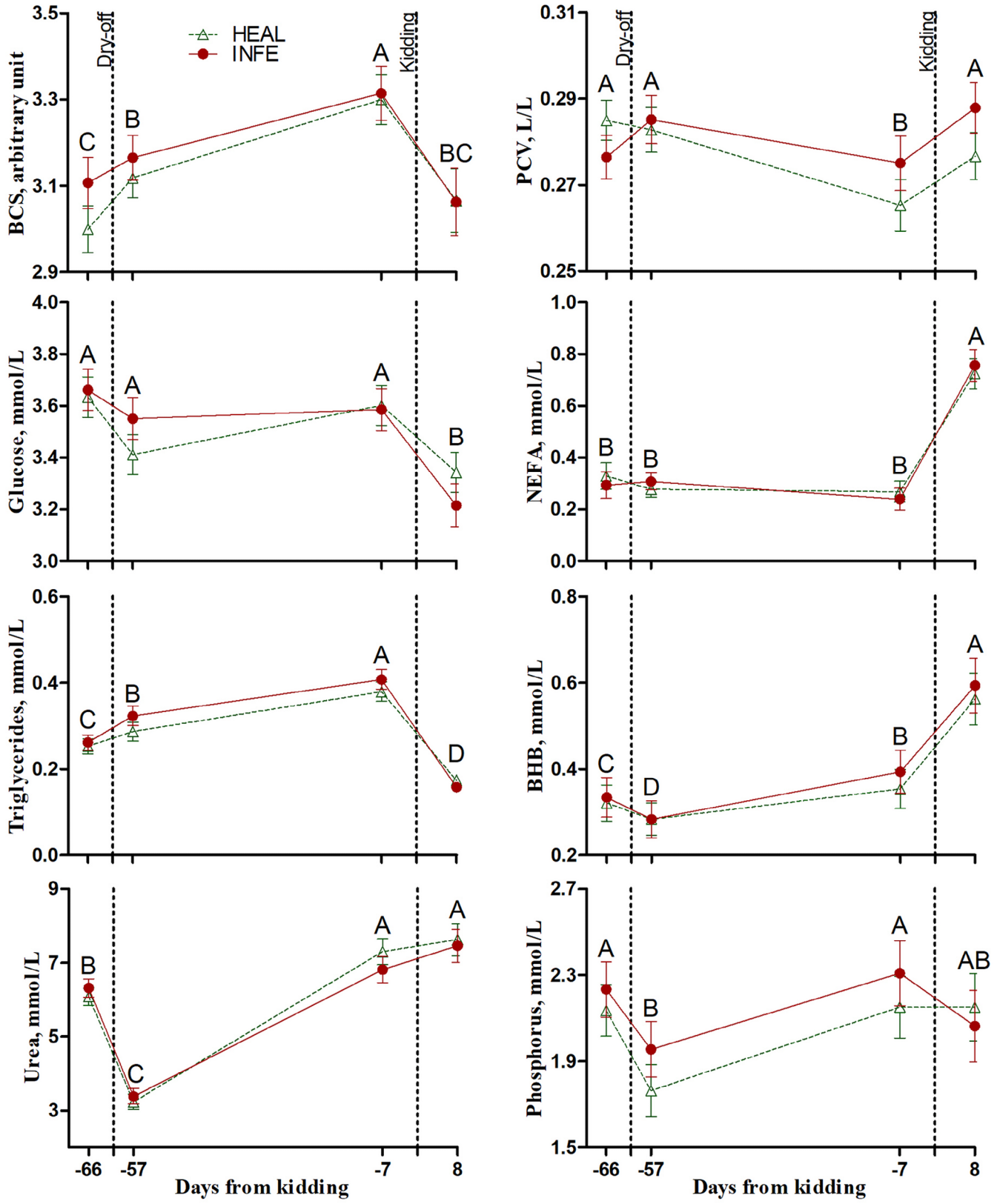

Figure 4. Time course of BCS, packed cell volume (PCV), and plasma concentrations of glucose, nonesterified fatty acids (NEFA), triglycerides, BHB, urea, and phosphorus in Alpine dairy goats classified as healthy (HEAL) or infected (INFE) based on milk samples collected 7 d before dry-off. Different letters (A–D) indicate time points differing from each other ($P < 0.05$).

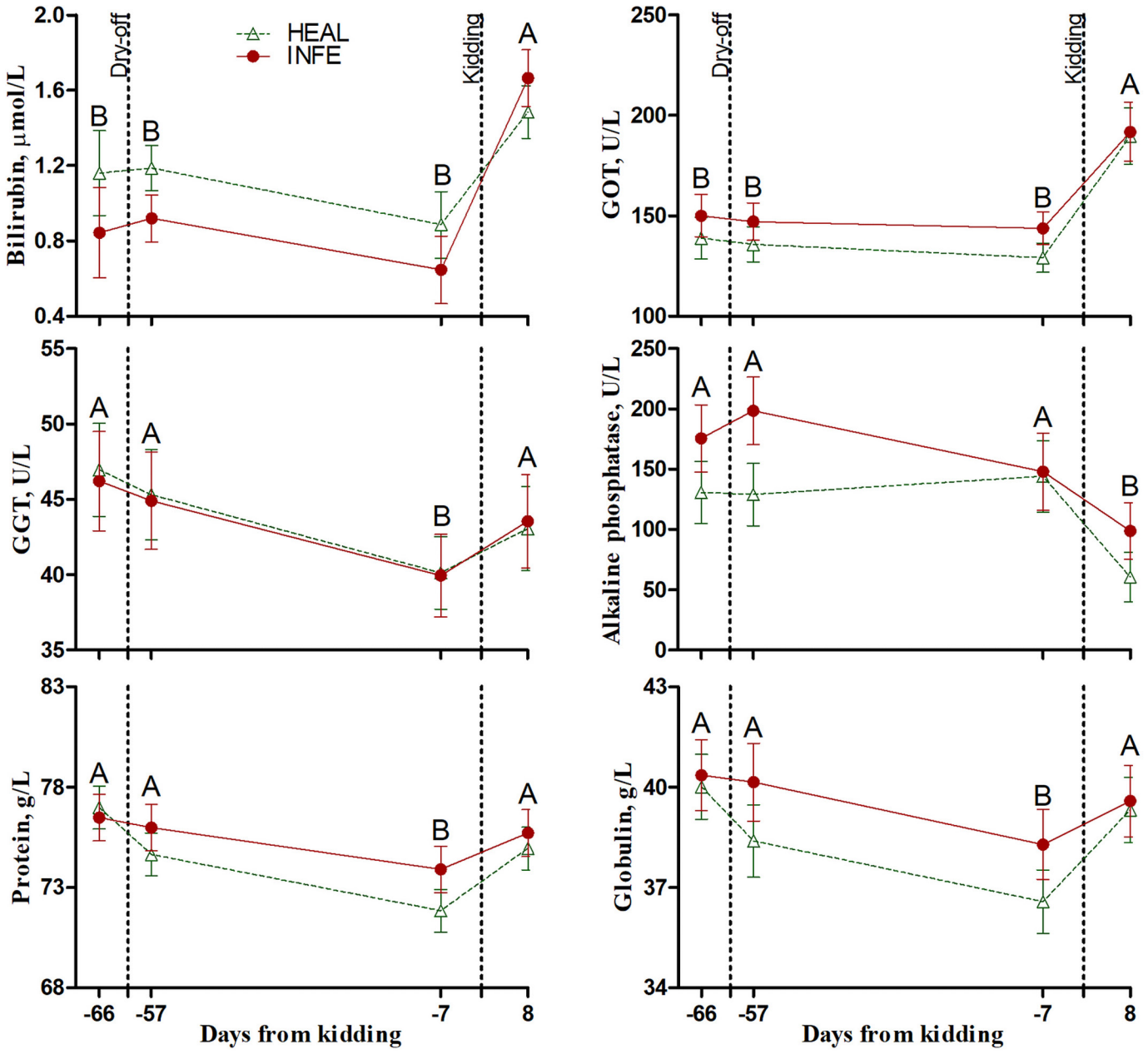

Figure 5. Time course of plasma concentrations of bilirubin, glutamate-oxalacetate transaminase (GOT), γ-glutamyl transferase (GGT), alkaline phosphatase, protein and globulin in Alpine dairy goats classified as healthy (HEAL) or infected (INFE) based on milk samples collected 7 d before dry-off. Different letters (A,B) indicate time points differing from each other ($P < 0.05$).

The involution of the mammary gland is a candidate trigger for APR trends observed prepartum. Evidence from dairy cows shows that inflammatory responses at dry-off can have systemic repercussions extending into the subsequent lactation (Cattaneo et al., 2021). In dairy goats, the process of mammary involution starts in the final stages of lactation, as reflected by the peak in SCC and SCS detected at −82 DFK in our goats, even reflecting the shedding of epithelial cells (Haenlein, 2002) and the infiltration of leukocytes into the mammary gland (Tedde et al., 2019). Concurrently, the observed drop in milk lactose may indicate increased mammary permeability, leading to lactose leakage into the bloodstream, a phenomenon well-documented in dairy cows during involution (Zhao et al., 2019). In this context, the post-dry-off peak in plasma myeloperoxidase—a well-established marker of neutrophil activation (Faith et al., 2008)—likely reflects immune activity within the mam-

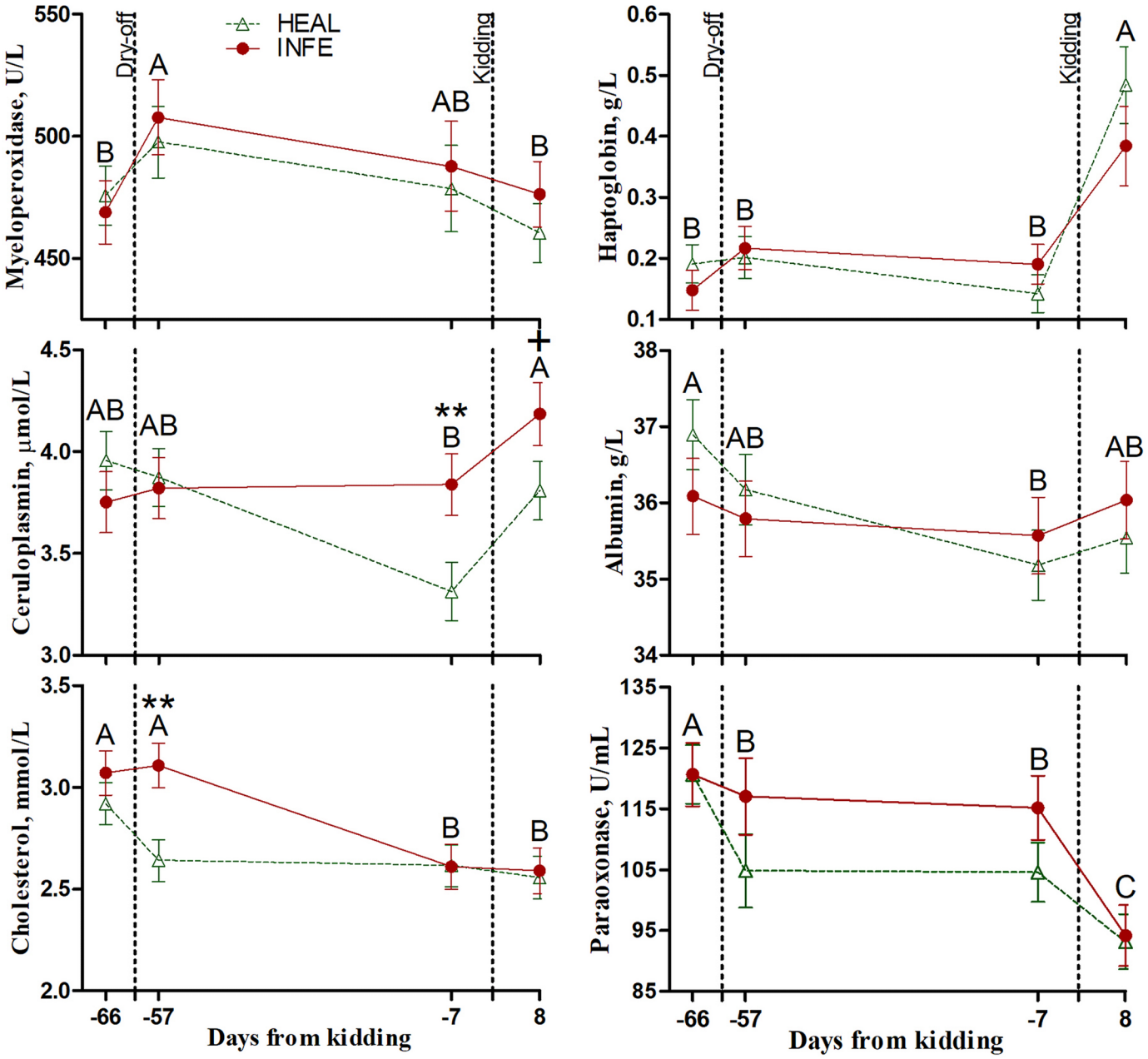

Figure 6. Time course of plasma concentration of myeloperoxidase, haptoglobin, ceruloplasmin, albumin, cholesterol and paraoxonase in Alpine dairy goats classified as healthy (HEAL) or infected (INFE) based on milk samples collected 7 d before dry-off. Letters (A–C) indicate time points differing each other ($P < 0.05$) and symbols reflect differences between HEAL and INFE group when the health status × time interaction is significant (HS × time; $^{**}P < 0.01$; $^{+}P < 0.1$).

mary gland. The sustained elevation in the MPR until 8 DFK in our study—a marker of systemic inflammation (Nedić et al., 2019)—supports the existence of a systemic inflammation triggered by dry-off that, compounded by the escalating metabolic demands of advanced pregnancy, possibly contributed to the APR biomarker patterns observed in our goats before parturition (Putman et al., 2018; Mezzetti et al., 2024).

The Health Status of the Mammary Gland Before Dry-Off Influenced Somatic Cell Count Trends and Some Plasma Analytes Related to Inflammation and Redox Balance

The goats included in the present study should be considered clinically healthy, as none exhibited signs of clinical mastitis or any other disease from 30 d before

enrollment onward. The results of the BC performed before dry-off identified *Staphylococcus caprae* and *Staphylococcus equorum* as the dominant species in INFE goats, with minor incidences of *Serratia marcescens* and *Staphylococcus aureus* being the only pathogenic bacterium detected (Hossain et al., 2025). Furthermore, the follow-up performed on BC at resumption of lactation (i.e., in colostrum and at 8 DFK) revealed a reduction in the prevalence of IMI in CG goats (Hossain et al., 2025). Finding reliable markers of subclinical mastitis in dairy goats is still debated, and the reliability of SCC in this respect remains unclear (Min et al., 2007). Although some studies report that BC positivity does not significantly affect SCC (Gocmen et al., 2019), others have found a significant correlation (Ying et al., 2004). In our study, INFE goats had higher SCC at −82 DFK and higher SCS at 45 DFK. Because SCS is the logarithmic transformation of SCC, its use reduces the high variability of cell counts observed in a single DHI test-day (Cassell, 1994). Thus, this normalization likely allowed better discrimination of the differences between groups of goats at mid-lactation, when SCC is more representative of leukocyte infiltration related to IMI. In plasma, INFE goats had higher concentrations of ROMt between −57 and 8 DFK, possibly reflecting increased generation of oxidant species by activated leukocytes (Robinson, 2009). This oxidative challenge could, in turn, explain the elevated thiol concentrations detected in the same group at −57 and −7 DFK, as reactive oxygen species are known to act as signaling molecules that stimulate the release of endogenous antioxidants (Celi and Gabai, 2015). The higher ceruloplasmin concentrations observed in INFE goats at −7 and 8 DFK indicate a more pronounced APR around kidding in these animals, supporting the hypothesis that compromised mammary health at dry-off exacerbates the inflammatory response at the subsequent parturition, as previously reported in dairy cows (Cattaneo et al., 2021). The peak in cholesterol observed in INFE goats after dry-off is more difficult to interpret. Although cholesterol is generally considered a negative acute phase protein indicator (even reflecting secretion of lipoproteins by the liver), and higher levels typically suggest a milder inflammatory state, similar findings have been reported in a murine model of *S. aureus*-induced mastitis, in which circulating cholesterol increased following the mammary challenge (Xiao et al., 2017). While the occurrence of transient dyslipidemia associated with bacterial IMI in goats remains speculative, this interpretation aligns with the cholesterol trends observed in our study. Thus, plasma concentrations of ceruloplasmin, cholesterol, ROMt, and thiols measured around dry-off and kidding mirrored the SCC patterns observed in the milk of INFE goats, suggesting that these analytes may serve as useful proxies for systemic immune activation in response to IMI. In contrast, BC classification did not affect milk yield or composition during the subsequent lactation, nor did it influence other plasma biomarkers associated with the APR and inflammation (i.e., haptoglobin, myeloperoxidase, albumin, and paraoxonase).

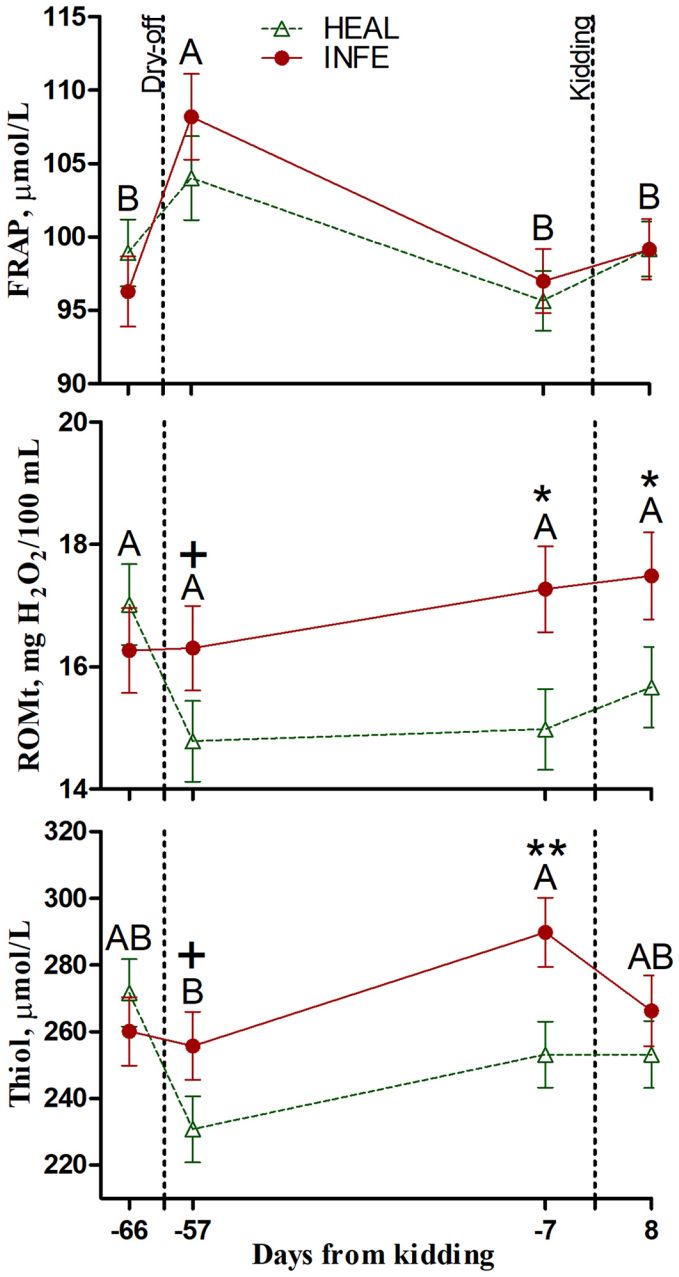

Figure 7. Time course of plasma concentration of ferric ion reducing antioxidant power (FRAP), total reactive oxygen species (ROMt), and thiol in Alpine dairy goats classified as healthy (HEAL) or infected (INFE) based on milk samples collected 7 d before dry-off. Different letters (A,B) indicate time points differing from each other ($P < 0.05$), and symbols reflect differences between HEAL and INFE group when the health status × time interaction is significant (HS × time; *$P < 0.05$; **$P < 0.01$; +$P < 0.1$).

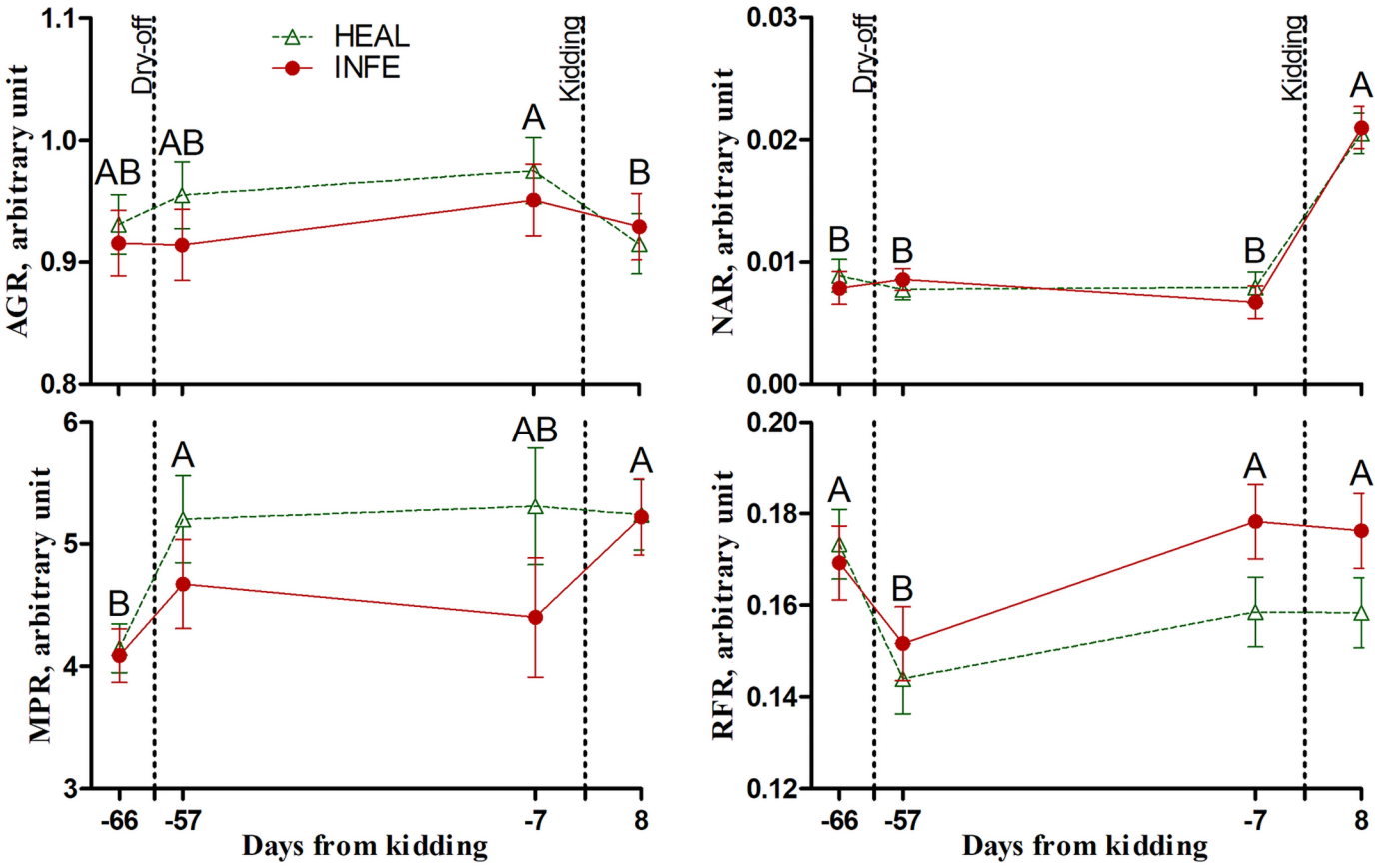

Figure 8. Time course of ratios between plasma concentrations of albumin and globulin (AGR), nonesterified fatty acids (NEFA) and albumin (NAR), myeloperoxidase and paraoxonase (MPR), and total reactive oxygen species and ferric ion reducing antioxidant power (RFR) in Alpine dairy goats classified as healthy (HEAL) or infected (INFE) based on milk samples collected 7 d before dry-off. Different letters (A,B) indicate time points differing from each other ($P < 0.05$).

Potential limitations of this study should be considered when interpreting these results. As this is a pilot study aimed at exploring the potential association between mammary health status and trends of plasma analytes detected around dry-off, it was not possible to perform a sample size calculation tailored to plasma analytes before study initiation. To address this, we performed a post hoc power analysis (PROC POWER, SAS) considering $\alpha = 0.05$ and $n = 30$. The results indicated sufficient statistical power for cholesterol (0.847), moderate power for ROMt (0.700), and lower power for ceruloplasmin and thiol (<0.500). Another limitation is the reliance on monthly DHI test-day records, in the absence of daily automated milk recording systems (generally unavailable in Italian goat farms), that restricted the resolution of productive data. Finally, a structural limitation to acknowledge is the use of BC as the sole udder health indicator to classify our goats. In dairy cows, BC positivity may occur without a detectable immune response, whereas some pathogens—particularly intracellular bacteria—can escape detection despite inducing subclinical mastitis. These limitations may partly explain why no effects of BC classification were observed on productive performance or certain plasma inflammatory biomarkers, and why no measurable impact of antibiotic treatment emerged, despite our previous work showing reduced IMI prevalence at the onset of lactation in CG goats (Hossain et al., 2025).

CONCLUSIONS

This pilot study documented changes in cholesterol, ceruloplasmin, ROMt, and thiol groups detected in Alpine dairy goats between dry-off and kidding to reflect systemic alterations associated with IMI. Even though their direct application in routine farm diagnostics will be limited, these plasma analytes could serve as promising proxies for detecting systemic responses to IMI, aiding the validation of novel candidate biomarkers for subclinical mastitis detection in goat milk. To be extended to field application, the relationship between these biomarkers and IMI status of Alpine goats needs to be further investigated in future studies with adequate

sample size and conducted across diverse production systems, and the information on the variability of plasma analytes between dry-off and kidding provided here can help design them more effectively.

NOTES

The authors thank Marinella Buzzini, Massimo Buzzini, Rachele Mezzetti, and Maria Paola Rossi for kindly allowing this experiment to be conducted on their farm (San Rocco al Porto, Italy). Special thanks are extended to veterinarians Mario Villa and Simone Fasana (Lombardia, Italy) for their valuable contribution and supervision during the trial. The authors are also grateful to Deatech SRL (Milan, Italy) for their support in formulating the diets, and to the Associazione Italiana Allevatori (AIA; Rome, Italy) for collecting and sharing data on the productive performances of the goats. Supplemental material for this article is available at https://doi.org/10.17632/mcp58yrh42.1. This study was conducted in accordance with Italian legislation on animal experimentation (Legislative Decree No. 116, 27/01/1992). The experimental design and procedures involving the goats were reviewed and approved by the Ethics Committee for Animal Welfare of the University of Milan, Italy (approval no. OPBA 118–2023), and were conducted in compliance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. The authors have not stated any conflicts of interest.

Nonstandard abbreviations used: AGR = albumin to globulin ratio; APR = acute phase response; BC = bacterial culture; CG = control group; DFK = days from kidding; FRAP = ferric ion reducing antioxidant power; GGT = gamma glutamyl transferase; GOT = glutamate oxaloacetate transaminase; HEAL = healthy goats; INFE = infected goats; HS = health status; KN = number of kids carried by the goat; MPR = myeloperoxidase to paraoxonase ratio; NAR = NEFA to albumin ratio; NEFA = nonesterified fatty acids; PAR = parity; PCV = packed cell volume; RFR = ROMt to FRAP ratio; ROMt = reactive oxygen metabolites; TG = treatment group; Treat = treatment; UFL = unité fourragère lait.

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