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Drying-off dairy cows without antibiotic therapy and orally supplemented with lyophilized *Aloe arborescens*: effects on rumen activity, immunometabolic profile, and milk yield

Luca Cattaneo[1] | Fiorenzo Piccioli-Cappelli[1] | Andrea Minuti[1] | Erminio Trevisi[1,2]

[1]Department of Animal Science, Food and Nutrition (DIANA), Faculty of Agricultural, Food and Environmental Sciences, Università Cattolica del Sacro Cuore, Piacenza, Italy

[2]Romeo and Enrica Invernizzi Research Centre for Sustainable Dairy Production (CREI), Università Cattolica del Sacro Cuore, Piacenza, Italy

Correspondence
Erminio Trevisi, Department of Animal Science, Food and Nutrition (DIANA), Faculty of Agricultural, Food and Environmental Sciences, Università Cattolica del Sacro Cuore, 29122 Piacenza, Italy.
Email: erminio.trevisi@unicatt.it

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Abstract

The drying-off is a stressful stage of the lactation cycle of dairy cows that deeply affects cows' metabolism, inflammatory status, and immune system. The promising effects observed during the transition period resulting from supplementation with *Aloe arborescens* Mill. suggest its potential utility during this phase. A group of 23 Holstein dairy cows with somatic cell count (SCC) less than 200×10^3 cells/ml and without intramammary infections were enroled in the study. Cows were divided into two groups: one orally receiving 10 g/day of *A. arborescens* Mill. lyophilized powder (AL; 11 cows) between −7 and 7 days from dry-off (DFD), and a control group (CTR; 12 cows). From −14 to 7 DFD and 7 and 28 days from calving, the body condition score and rectal temperature were determined, and rumen fluid, feces, milk, and blood samples were collected. Daily rumination times and milk yield were recorded. Data were analyzed through repeated measures mixed models. Compared to the CTR group, AL cows tended to show reduced production of volatile fatty acids in the rumen with acetate proportion that tended to be higher and valerate proportion that was lower. Moreover, *Aloe* supplementation caused a reduction in fecal dry matter. At the end of drying-off, AL cows presented better liver function, as suggested by higher paraoxonase plasma concentrations at 7 DFD, higher glucose, and lower urea, but showed increased reactive oxygen metabolites. *Aloe* supplementation at dry-off ameliorated inflammatory status after calving (lower haptoglobin and ceruloplasmin levels), and improved milk yield in the first weeks of subsequent lactation, without influencing milk composition, SCC, and incidence of intramammary infections. These results confirmed the positive effects of *Aloe* administration on liver function in dairy cows but indicate the need for further studies investigating the effects of *Aloe* on rumen fermentation profile and oxidative status.

KEYWORDS
nutraceutical, rumen fermentation, selective dry-cow therapy, teat sealant

1 | INTRODUCTION

The dry-off is a challenging event of the lactation cycle in dairy cows. The transition from lactation to the dry period together with abrupt milking cessation, regrouping, and dietary changes, leads to a significant physiological imbalance (von Keyserlingk et al., 2008; Zobel et al., 2015). In fact, dry-off is a stressful event that causes impairment of metabolism, liver function, and antioxidant system and also triggers inflammatory responses (Bertulat et al., 2013; Cattaneo et al., 2021b; Mezzetti et al., 2020b). In particular, after dry-off, the non-esterified fatty acids (NEFA) and β-hydroxybutyric acid (BHB) increase due to the lower energy content of the diet (Putman et al., 2018) can harm immune system function (Lacetera et al., 2004). Moreover, mammary gland starts a period of intense remodelling, known as active involution (Hurley, 1989). During this remodelling phase, which begins 2 days after dry-off and lasts approximately 21 days, susceptibility to new intramammary infections is high (Bradley & Green, 2004; Eberhart, 1986). In addition, social pressure to decrease the use of antibiotics in livestock with the aim of limiting antibiotic resistance is increasing (ECDC/EFSA/EMA, 2017; Trevisi et al., 2014). In light of this decrease, the use of selective dry-cow therapy is increasing, but cows that are not treated with antibiotic therapy at dry-off could be at a higher risk of developing new infections during the mammary remodelling phase (Scherpenzeel et al., 2014; Winder et al., 2019). However, recent evidence showed that selective dry-cow therapy can effectively reduce the use of antimicrobials at dry-off without jeopardising udder health or milk production at the onset of lactation (Kabera et al., 2021; Weber et al., 2021).

Additionally, in cows with still high milk yield at dry-off, all these processes are exacerbated (Mezzetti et al., 2020b; Rajala-Schultz et al., 2005; Silanikove et al., 2013), and many strategies to face these issues have been proposed (Vilar & Rajala-Schultz, 2020). Most interventions that have been commonly studied and applied are related to dietary changes (Odensten et al., 2007; Ollier et al., 2014), reduction in milking frequency (Gott et al., 2017; Martin et al., 2020; Rajala-Schultz et al., 2018), or a combination of both (Dancy et al., 2019; Tucker et al., 2009). An alternative option could be represented by the administration of nutraceuticals (Bertoni et al., 2015, 2016). These products have been shown to produce positive effects on regulating immune responses and metabolism during the transition period (Lopreiato et al., 2020), but literature addressing the application of these products at dry-off is scarce.

Aloe spp. plants have been used for ages in traditional medicine for their therapeutic properties, such as wound healing, anti-inflammatory, antioxidant, antitumor, antimicrobial, and immunomodulatory effects (Singab et al., 2015). The most relevant species are *Aloe barbadensis* Mill. (also known as *A. vera*) and *Aloe arborescens* Mill. (Liao et al., 2006). *Aloe* leaves are rich in anthraquinones and their glycosides, found in the green rind, whereas the parenchyma is abundant in complex carbohydrates (Hamman, 2008). Although the effects of *Aloe* are likely due to a synergic activity of several compounds (e.g., phenols, polysaccharides, and vitamins), its main active compounds are the anthraquinones aloin A and B (also known as barbaloin) and acemannans (Pellizzoni et al., 2012). Anthraquinones have antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* (Hamman, 2008), and acemannans might have an indirect antimicrobial activity because they stimulate phagocytic leukocytes (Pugh et al., 2001).

Previous research demonstrated that aloin A is detectable in the blood of dairy cows as early as 2 h after oral administration of 200 g/day of whole leaves *A. arborescens* homogenate (obtained from 3 years plants) frozen preserved and thawed just before the use (Bani et al., 2016). In the same study, the authors did not observe any adverse effects resulting from *A. arborescens* administration on feed intake, digestibility, and rumen fermentation. In a subsequent experiment, Mezzetti et al. (2020a) fed in the same way 200 g/day of *A. arborescens* homogenate to dairy cows during the transition period. In this study, positive effects of *Aloe* on lipid mobilisation, hepatic and renal function, and on mitigating the inflammatory responses typical of the calving event were detected. However, the storage, preservation, and administration of homogenate are impractical in larger settings, and lyophilization of the homogenate can address these issues.

We hypothesised that *Aloe* could improve the transition from lactation to the dry period in dairy cows due to this plant's anti-hyperlipidemic and anti-inflammatory effects, particularly in subjects that did not receive antibiotic therapy during this phase. Thus, in cows treated with internal teat sealant alone at dry-off, we evaluated the effects of 10 g/day of lyophilized *A. arborescens* Mill. powder supplementation from −7 to 7 days from dry-off (DFD) on rumen function, milk production, somatic cell count (SCC), and hematological biomarkers in dairy cows at dry-off and during the subsequent lactation.

2 | MATERIALS AND METHODS

2.1 | Animal management and experimental design

The research was conducted at Università Cattolica del Sacro Cuore dairy barn (Cerzoo, San Bonico, Piacenza, Italy) in accordance with Italian laws on animal experimentation and ethics (Italian Health Ministry Authorisation No. 444/2019-PR in agreement with D. Lgs. no. 26, 04/03/2014). Fourteen days before dry-off (−14 DFD), sterile milk from each quarter and composite milk were collected for bacteriological and SCC analysis respectively. Cows with SCC less than 200×10^3 cells/ml and without intramammary infections due to major pathogens were included in the study. A group of 23 Holstein dairy cows (parity expressed as mean ± standard deviation [*SD*] 2.43 ± 1.34), was enroled in the study. During lactation, cows were housed in a free-stall pen, received lactation diet, and were milked twice daily (5:00 and 17:00 h). About 55 days before expected calving, cows were abruptly dried off with the infusion of internal teat sealant (Noroseal, Norbrook Laboratories Limited, Newry, UK) after the last milking. The average milk yield during the week leading to dry-off was 22.7 ± 5.7 kg/day (mean ± *SD*). Afterwards, they were moved to a dry pen and received only grass hay for 7 days after

which a diet for dry cows was administered. Rations were formulated according to the National Research Council (NRC, 2001) guidelines, and chemical composition of the rations is reported in Table 1. Regular checks were performed during the study period and several samples were collected according to the schedule shown in Figure 1 and described below.

TABLE 1 Ingredients and chemical composition of diets served as TMR during the study

	Dry cows	Lactating cows
Ingredient, % of DM		
Corn silage	11.6	32.6
Alfalfa hay	–	24.9
Corn ground	–	13.8
Soybean meal	4.8	11.4
Barley ground	–	9.2
Wheat silage	47.5	3.3
Sunflower meal	5.1	2.0
Mineral and vitamin	0.9	1.9
Hydrogenated fat	–	0.9
Straw	17.7	–
Grass hay	12.4	–
Chemical composition		
NE_L, Mcal/Kg	1.28	1.65
CP, % of DM	12.7	16.6
NSC, % of DM	9.7	30.0
NDF, % of DM	57.0	33.4
Calcium, % of DM	0.45	0.79
Phosphorus, % of DM	0.34	0.42

Abbreviation: TMR, total mixed ration.

Cows were selected for supplementation with 10 g/day of lyophilized *A. arborescens* Mill. whole leaves homogenate from −7 to 7 DFD (AL; $n = 11$) or none for the control group (CTR; $n = 12$). Groups were balanced for parity, previous lactation length, and SCC history. The dose of *Aloe* was calculated to provide similar DM amount as in the study of Mezzetti et al. (2020a). Since 200 g/day of homogenate *Aloe* with a DM content of about 7% were used (Bani et al., 2016), to provide a similar amount of DM we chose to use 10 g/day. Before total mixed ration (TMR) distribution, feed bunk was cleaned, all cows were restrained in the headlocks at distance to avoid cross-feeding, and each dose of lyophilized *Aloe* was mixed with 1 kg of lactation TMR and fed to AL cows, whereas CTR cows received only 1 kg of lactation TMR. During the whole trial, an operator checked that the cows ate all the supplemented TMR, and no leftovers were ever found so the treatment had to be administered in another way.

2.2 | Aloe processing and aloin determination

A. arborescens Mill. whole leaves (Dester Gardens, Crociale di Manerba del Garda, Italy) were cut and homogenised as reported by Bani et al. (2016). Briefly, whole leaves were cut and homogenised by a vegetable cutter (model R6; Robot Coupe). A sample was collected, and the homogenate was immediately frozen in plastic bags, with no additives. Afterward, all the homogenate was lyophilized at the same time, through evaporation of water content at −5 mbar at an increasing temperature from −40°C to 25°C (Biostarters S.r.l.). Lyophilizate was aliquoted and stored in the dark until use. Aloin content was determined using liquid chromatography coupled to triple quadrupole mass spectrometry via an electrospray ionisation source (LC-ESI/MS/MS), as previously described (Lucini et al., 2013, 2015).

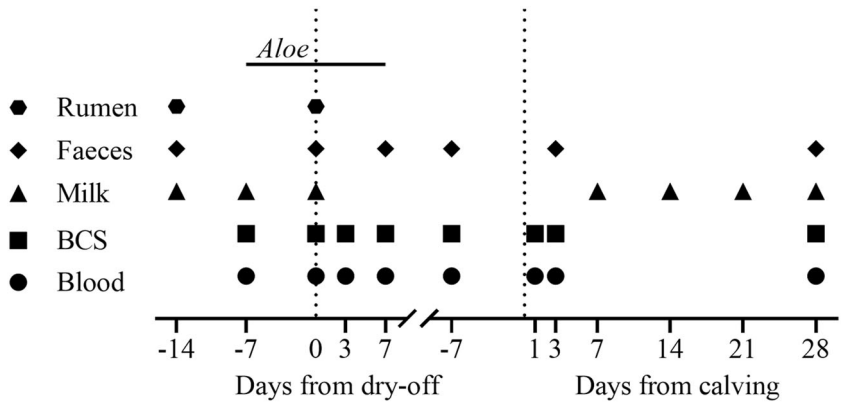

FIGURE 1 Study design reporting the scheduled time points of the different sampling procedures carried out. *Aloe arborescens* Mill. lyophilized powder was supplemented from −7 to 7 days from dry-off (DFD). Blood samples (together with BCS evaluation) were collected at −7, 0, 3, 7 DFD and −7, 1, 3, 28 days from calving (DFC); milk samples were collected at −14, −7, 0 DFD and 7, 14, 21, 28 DFC; fecal samples at −14, 0, 7 DFD and −7, 3, 28 DFC; rumen samples at −14 and 0 DFD

2.3 | Rumen fluid and fecal samples

At −14 and 0 DFD, rumen fluid samples were collected before the morning feeding using a ruminal probe specially designed for cattle (Ruminator; profs-products.com), as described by Wallace et al. (2019). To avoid salivary contamination, the first liter of collected rumen fluid was discarded, and the next 0.5 L was retained. For all samples, the pH of the rumen fluid was recorded immediately with a pH metre (GLP 21; Crison Instruments SA). Afterward, samples were gently mixed, and five aliquots of 1 ml each were pipetted into 2 ml tubes and immediately stored at −20°C for volatile fatty acid (VFA) measurements. Concentrations of VFA and D- and L-lactic acid were determined as described by Minuti et al. (2014). At −14, 0, and 7 DFD and −7, 3, and 28 days from calving (DFC), fecal samples were collected manually in plastic bags from the rectal ampulla immediately after rumen sampling. For all samples, pH was determined on fresh material after mixing, as the mean of six readings in different points of the. Fecal dry matter was determined from 200 g of fresh material after 96 h in a ventilated oven at 65°C.

2.4 | Blood samples and immunometabolic profile

Blood samples were collected from the jugular vein into heparinized tubes before the morning feeding on −7, 0, 3, and 7 DFD and −7, 1, 3, and 28 DFC. Tubes were immediately cooled in a water bath containing ice. An aliquot of whole blood was centrifuged to determine packed cell volume, while the remaining sample was centrifuged at 3500*g* for 15 min at 4°C. Afterwards, plasma was stored at −20°C for use in subsequent assays. Several parameters were analyzed using a clinical auto-analyzer (ILAB 650; Instrumentation Laboratory) as reported by Calamari et al. (2016): calcium, phosphorus, magnesium, zinc, glucose, cholesterol, urea, ceruloplasmin, total protein, albumin, globulin, aspartate-aminotransferase (AST-GOT), γ-glutamyl transferase, alkaline phosphatase, bilirubin, haptoglobin, NEFA, BHB, creatinine, paraoxonase, myeloperoxidase, total reactive oxygen metabolites (ROM), thiol groups, advanced oxidation protein products (AOPP), ferric reducing antioxidant power (FRAP), retinol, tocopherol, and β-carotene. Albumin and globulin concentrations were used to calculate the albumin-to-globulin ratio, the liver functionality index (LFI) was calculated according to Bertoni and Trevisi (2013), and the oxidative status index (OSI) was calculated as the ratio between ROM and FRAP.

2.5 | Body condition score, rectal temperature, health status, and rumination time

At the time of blood sampling, the body condition score (BCS) was evaluated by the same operator using a 1- to 4-point scale (ADAS, 1986), and body temperature was measured with a digital thermometer. Health status was monitored, and diseases and their corresponding detection dates were registered. Mastitis was diagnosed by visual evaluation of abnormal milk from each quarter and SCC analysis on suspicious cases. Rumination time was recorded daily with the Hr-LD tags (SCR by Allflex) from −7 to 14 DFD, and from −14 to 28 DFC.

2.6 | Milk yield and composition

Milk yields were automatically recorded at each milking session in the parlour and expressed as a weekly average. At −14, −7, and 0 DFD and 7, 14, 21, and 28 DFC, approximately 100 ml of milk were collected for the determination of milk composition and SCC. Milk composition (fat, protein, lactose, casein, urea) was using a Milkoscan FT120 analyzer (Foss Analytics, Hillerød, Denmark), and SCC by Fossomatic 180 (Foss Analytics). SCC was expressed as linear score (SCS; Wiggans & Shook, 1987).

At −14, −7, and 0 DFD and 7, 21, and 28 DFC, milk samples were aseptically collected from each quarter and analyzed for bacterial contamination within 24 h of the collection as described in Cattaneo et al. (2021c). Samples at −14 DFD were used to enrol the cows in the trial, to avoid the presence of pathogens that made compulsory antibiotic treatment. In subsequent samples, carried out to provide information about intramammary infection status through the experiment, those tested positive for *Staphylococcus aureus*, *Streptococcus uberis*, *Enterobacter* spp., *Escherichia coli*, *Trueperella pyogenes*, and *Serratia marcescens* were considered infected by major pathogens, whereas samples tested positive for coagulase-negative staphylococci and *Corynebacterium bovis* were considered infected by minor ones. Samples positive for more than three different bacterial species were considered contaminated.

2.7 | Statistical analysis

Sample size was calculated based on the expected anti-hyperlipidemic and anti-inflammatory effect of *Aloe* supplementation, considering NEFA and haptoglobin as the primary outcomes. From previous investigations in this phase (Cattaneo et al., 2021a), the required sample size in a repeated measures design to achieve a statistical power of 0.8 with $\alpha = 0.05$ and an expected effect size of 0.4 in NEFA levels was 11 subjects per group (G*Power package; Faul et al., 2007). A similar sample size was required according to haptoglobin.

Data were analyzed with SAS software, version 9.4 (SAS Institute). Rumination time, milk, feces, and plasma parameter data were subject to analysis of variance testing using a mixed model for repeated measures (GLIMMIX Procedure; SAS Institute). The statistical model included fixed effects of the treatment (TRT) for CTR and AL groups, time (T), and interactions of the two factors (TRT × T), and cows as random effects. In the milk yield model, mature equivalent milk yield in the previous lactation was included in the model as a covariate. Covariance structures

(compound symmetry, autoregressive order, and spatial power) were included in the model according to the Akaike information criterion with the one having the lowest criterion being chosen (Littell et al., 1998). Plasma biomarkers and rumination times around dry-off and calving were analyzed separately to evaluate the effects in these two distinct phases. Rumen fluid analysis performed at 0 DFD was subject to analysis of covariance testing (GLM Procedure; SAS Institute) with the use of baseline (−14 DFD) as a covariate (Bland & Altman, 2015) after considering only the fixed effect of treatment. The pairwise comparison was done using the Tukey test. Post-hoc comparisons were discussed when $p \leq 0.05$. The main effects at $p \leq 0.10$ were discussed as tendencies.

3 | RESULTS

The main characteristics of the two groups of cows at the enrolment in the experiment are reported in Table 2. Overall, groups were balanced with respect to the recruitment of cows with no relevant differences between them, except for milk yield in the previous lactation resulting numerically higher in AL cows. *Aloe* homogenate's aloin content was 4.6 ± 0.5% (mean ± *SD*; on a DM basis), whereas lyophilizate had 1.3 ± 0.4% DM of aloin.

3.1 | Rumen fluid, feces, and rumination time

Rumen fluid pH was not affected by *Aloe* treatment (Table 3). Otherwise, as shown in Table 3, the treatment affected the VFA production at 0 DFD. Compared with the CTR group, the AL group tended to have greater proportion of acetate ($p = 0.06$) and a lower valerate proportion ($p = 0.03$) was detected. Concentrations of ammonia, D- and L-lactate, and other VFA proportions were not affected by *Aloe* treatment. Daily rumination time did not differ between groups but varied with time both at drying-off and at calving (T; $p < 0.01$; Figure 2).

Fecal pH did not differ between groups both at dry-off and calving (Table 4), whereas a tendency toward an effect of treatment was observed in fecal dry matter with lower values observed in AL versus CTR cows, both at dry-off and calving ($p = 0.08$ and 0.07, respectively).

3.2 | Udder health

The presence of pathogens in the udder before dry-off and in the first month of lactation is shown in Table 5. Incidence of mastitis in the subsequent lactation did not differ between groups (36% vs. 25% for AL and CTR, respectively; $p = 0.55$).

Before dry-off and after calving, SCS did not differ between groups (Table 6). On average, during the whole lactation period, no differences were detected between groups (2.78 ± 0.37 vs. 2.12 ± 0.35 for AL and TS, respectively; $p = 0.21$).

TABLE 2 Main characteristics at the enrolment of dairy cows in the control group (CTR) or receiving 10 g/day of lyophilized *Aloe arborescens* Mill. (AL) from −7 to 7 days relative to dry-off

Item, unit	Treatment		SEM[a]	*p* value[b]
	AL	CTR		
Parity, n	2.5	2.3	0.4	0.71
Mature equivalent milk production, kg	12,317	11,539	373	0.15
Lactation length, d	332	352	15.0	0.35
Average somatic cell count, SCS[a]	2.08	1.96	0.29	0.77
Dry period length, d	50.3	51.9	2.5	0.64
Milk yield at dry-off, kg/d	24.2	21.4	1.7	0.26
Somatic cell count at dry-off, SCS[c]	2.61	2.27	0.29	0.41

[a]SEM = greatest standard error of the mean.

[b]*p* value of the treatment effect.

[c]SCS = Somatic Cell Score (Wiggans & Shook, 1987).

TABLE 3 Rumen fluid pH and concentration of ammonia, D-lactate, L-lactate, total volatile fatty acids (VFA), and VFA proportions (%, mmol/100 mol of VFA) on the day of dry-off in dairy cows in the control group (CTR) or receiving 10 g/day of lyophilized *Aloe arborescens* Mill. (AL) from −7 to 7 days relative to dry-off

Item, unit	Treatment		SEM[a]	*p* value[b]
	AL	CTR		
pH	6.88	6.76	0.08	0.30
Ammonia, mmol/L	18.0	19.0	1.8	0.72
D-lactate, mmol/L	217	191	49	0.71
L-lactate, mmol/L	217	193	48	0.72
Total VFA production, mmol/L	89.2	101.3	4.8	0.08
VFA proportion, %				
Acetate	65.7	64.0	0.6	0.06
Propionate	18.5	20.0	0.7	0.13
Butyrate	11.0	11.3	0.4	0.55
Isobutyrate	1.19	1.11	0.06	0.40
Valerate	1.14	1.28	0.04	0.03
Isovalerate	1.86	1.81	0.08	0.68

[a]SEM = greatest standard error of the mean.

[b]*p* value of the treatment effect adjusted for baseline (−14 days from dry-off).

3.3 | Milk yield and composition

Milk yield in the first 30 weeks of lactation is shown in Figure 3. Cows receiving AL at dry-off tended to have higher milk yield, particularly during the second month of lactation (weeks 4–6 after calving;

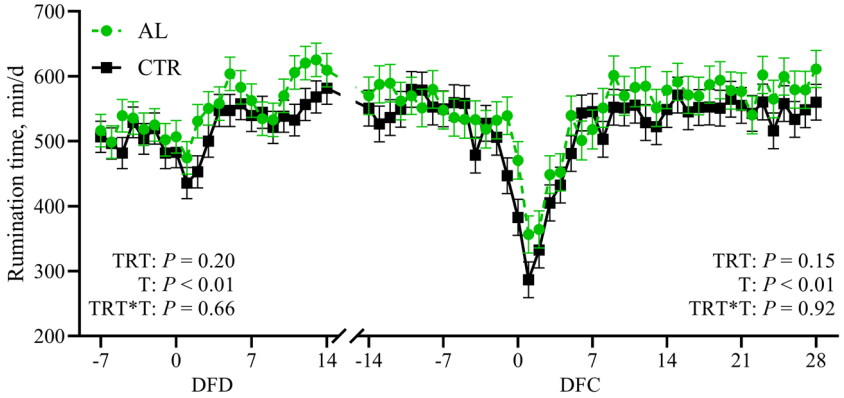

FIGURE 2 Daily rumination time around dry-off (from −7 to 14 days from dry-off [DFD]) and calving (from −14 to 28 days from calving [DFC]) in dairy cows receiving 10 g/day of lyophilized *Aloe arborescens* Mill. (AL) from −7 to 7 days relative to dry-off or in the control group (CTR). Values presented as least squares means ± standard error. In the text box, *p* values for the main effect of treatment (TRT), time (T), and interaction of treatment and time (TRT × T) are shown [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 4 Dry matter and pH of feces collected around dry-off (−14, 0, and 7 days relative to dry-off) and calving (−7, 3, and 28 days relative to calving) in dairy cows in the control group (CTR) or receiving 10 g/day of lyophilized *Aloe arborescens* Mill. (AL) from −7 to 7 days relative to dry-off

	Treatment			*p* value[b]		
Item, unit	AL	CTR	SEM[a]	TRT	T	TRT × T
Around dry-off						
Fecal pH	6.51	6.49	0.05	0.79	<0.01	0.63
Fecal dry matter, %	12.80	13.45	0.26	0.08	0.05	0.32
Around calving						
Fecal pH	6.44	6.47	0.05	0.61	<0.01	0.43
Fecal dry matter, %	12.69	13.53	0.32	0.07	0.14	0.06

[a]Greatest standard error of the mean.

[b]*p* value of main effects: treatment (TRT), time (T), and interaction between treatment and time (TRT × T).

$p < 0.1$), resulting in an interaction effect (TRT × T; $p < 0.01$). In Table 6, milk composition during the last week of lactation and in the first month after calving is reported. Except for urea, all analyzed variables varied with time after calving ($p < 0.01$), but not before dry-off. *Aloe* treatment did not lead to any difference between groups in the periods investigated.

3.4 | BCS, rectal temperature, and immunometabolic profile

Despite significant variations during the study period, BCS and rectal temperature did not present any difference between groups.

Dry-off caused significant alterations in most of the plasma biomarkers ($p \leq 0.05$), except for AOPP, haptoglobin, thiol groups, and albumin (Table S1). *Aloe* administration at dry-off had a significant effect on plasma concentrations of glucose, urea, paraoxonase, and ROM around the drying-off period (Figure 4). Glucose increased more in AL than in the CTR group starting from 0 DFD (TRT × T; $p = 0.03$), whereas urea concentration tended to be lower in AL after dry-off (TRT; $p = 0.07$). Paraoxonase and ROM concentration increased more in AL group starting from fry-off (TRT × T; $p = 0.01$ and 0.04, respectively). Other plasma biomarkers and OSI did not show any differences between groups around dry-off.

Around calving, all tested plasma biomarkers varied with time ($p < 0.01$; Table S2). *Aloe* treatment at dry-off tended to influence biomarkers of inflammation levels (Figure 5). The peak in haptoglobin concentration after calving was lower in the AL than in the CTR group (3 DFC; $p = 0.04$), resulting in a tendency towards an interaction effect (TRT × T; $p = 0.08$). Ceruloplasmin levels tended to be higher in the CTR group around calving (TRT; $p = 0.09$). Other plasma biomarkers, OSI, and LFI (1.28 ± 0.76 and 0.63 ± 0.73 points for AL and TS, respectively; $p = 0.54$) did not differ between groups around calving.

4 | DISCUSSION

Aloe is a plant known worldwide for its therapeutic use. In particular, *A. arborescens* leaves are rich in aloin, and aloenin, contain a good amount of polyphenols and flavonoids, and showed antioxidant activity (Cardarelli et al., 2017; Lucini et al., 2015). Remarkably, among *Aloe* species, *A. arborescens* has the highest total phenolic concentration and total antioxidant activity (Zapata et al., 2013). In a previous paper, Bani et al. (2016) described the use of *A. arborescens* Mill. homogenate as a nutraceutical in dairy cattle. In fact, the authors demonstrated that aloin, one of the main *Aloe* active compounds, is successfully absorbed into the blood. Moreover, no side effects of the *Aloe* on feeding behaviour and health were reported. Afterward, Mezzetti et al. (2020a) tested the supplementation of 200 g/day of

TABLE 5 Distribution of quarters at dry-off and calving in dairy cows in the control group (CTR) or receiving 10 g/day of lyophilized *Aloe arborescens* Mill. (AL) from −7 to 7 days relative to dry-off

	Before dry-off		After calving	
	AL (*n* = 125)	CTR (*n* = 112)	AL (*n* = 94)	CTR (*n* = 107)
Uninfected	94%	96%	94%	92%
Infected with major pathogens[a]	1%	1%	2%	3%
Infected with minor pathogens[b]	5%	3%	4%	6%

[a]Staphylococcus aureus, Streptococcus uberis, Enterobacter spp., Escherichia coli, Trueperella pyogenes, Serratia marcescens.

[b]Coagulase-negative staphylococci and *Corynebacterium bovis*.

TABLE 6 Average milk composition before dry-off (−7 and 0 days relative to dry-off) and after caving (7, 14, 21, and 28 days relative to calving) in dairy cows in the control group (CTR) or receiving 10 g/d of lyophilized *Aloe arborescens* Mill. (AL) from −7 to 7 days relative to dry-off

	Treatment			*p* value[b]		
	AL	CTR	SEM[a]	TRT	T	TRT × T
Before dry-off						
Fat, mg/100 ml	5.02	4.72	0.27	0.42	0.23	0.75
Protein, mg/100 ml	3.87	3.95	0.11	0.60	0.11	0.24
Casein, mg/100 ml	2.91	2.98	0.09	0.60	0.07	0.17
Lactose, mg/100 ml	4.96	4.91	0.08	0.66	0.41	0.78
Somatic cell count, SCS[c]	2.51	2.35	0.27	0.67	0.98	0.31
After calving						
Fat, mg/100 ml	4.28	4.45	0.21	0.58	<0.01	0.99
Protein, mg/100 ml	3.57	3.55	0.08	0.83	<0.01	0.52
Casein, mg/100 ml	2.63	2.62	0.06	0.83	<0.01	0.52
Lactose, mg/100 ml	5.03	5.01	0.05	0.76	<0.01	0.41
Somatic cell count, SCS[c]	2.27	2.49	0.42	0.71	<0.01	0.51

[a]Greatest standard error of the mean.

[b]*p* value of main effects: treatment (TRT), time (T), and interaction between treatment and time (TRT × T).

[c]SCS = Somatic Cell Score (Wiggans & Shook, 1987).

Aloe homogenate in transition dairy cows and obtained promising results. They added supplemental *Aloe* for 2 weeks before and after calving and observed positive effects on liver and kidney functions. Those effects could have been related to the anti-hyperlipidemic and anti-inflammatory effects of *Aloe*. Moreover, an improvement in antioxidant status in early lactation was also found, which likely mitigated the liver dysfunction typical of this physiological phase (Bertoni et al., 2008). Therefore, since the dry-off represents another critical and potentially stressful transition for dairy cows (Cattaneo et al., 2021b; Mezzetti et al., 2020b; Zobel et al., 2015), we evaluated whether *Aloe* supplementation for 7 days before and after dry-off could have positive outcomes also in this phase and if this could affect the subsequent early lactation period. However, the processing and delivery of *Aloe* homogenate are time-consuming and not easily implantable on a larger scale. Lyophilization has shown the best results in preserving the chemical quality of Aloe (Pawłowicz et al., 2021; Saravanan et al., 2015). Therefore, to facilitate easier administration of this plant, in this trial we lyophilized its homogenate, allowing also a more efficient storing and the possibility of feeding it together with the ration. The dry matter of lyophilized *Aloe* provided in the present study was comparable to that used in previous studies (Bani et al., 2016; Mezzetti et al., 2020a, 2020b), but aloin content was lower (1.3 vs. 2.4% of DM). The lyophilization process or differences in the raw material might account for this reduction. *Aloe* properties are dependent on the species, growing conditions, extraction, and preservation methods. Similar aloin concentration was obtained in *A. arborescens* lyophilized leaf extract by other authors (2.0%; Froldi et al., 2019). Moreover, many properties of *Aloe* are linked also to its phenolic components, which are preserved with this processing procedure (Froldi et al., 2019; Lucini et al., 2015).

In the current study, supplementation with lyophilized *Aloe* whole leaves did not alter rumen fluid pH but affected VFA proportions. Although it is known that nutraceuticals through their essential oils can modify rumen fermentation (Calsamiglia et al., 2007), Bani et al. (2016) reported a lack of effects of *Aloe* homogenate on in vitro fermentation parameters even though *Aloe* tended to cause an increase in total VFA production. With regards to molar proportions, acetate tended to increase after 1 week of lyophilized *Aloe* supplementation when compared with the control, while valerate tended to decrease. Effects on rumen VFA due to *Aloe* administration that are reported in literature are inconsistent. Total VFA production increased in all previously reported trials, but effects on individual VFA varied. Calabrò et al. (2013) recorded an increase in acetate and a tendency for propionate and butyrate to decrease after testing in vitro dried leaves of *A. arborescens*. Singh et al. (2021) reported an increase in acetate and propionate when *A. barbadensis* waste was fermented in vitro, whereas Bani et al. (2016) did not observe any effect on these VFA after adding *A. arborescens* homogenate to rumen juice in vitro. These differences compared with the current study can be due to diets, different species, doses tested, time of sampling, and techniques used to manipulate the plant. Moreover, all of the previous studies analyzed VFA production

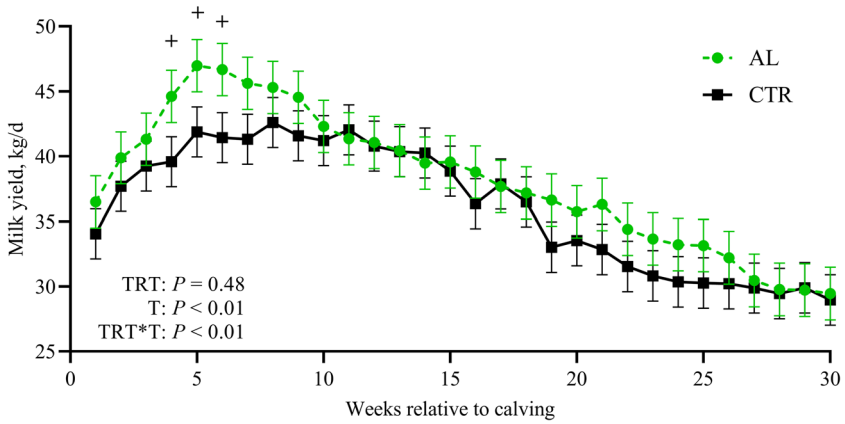

FIGURE 3 Milk yield in the first 25 weeks after calving in dairy cows receiving 10 g/day of lyophilized *Aloe arborescens* Mill. (AL) from −7 to 7 days relative to dry-off or in the control group (CTR). Values presented as least squares means ± standard error. Differences between groups within each time point are denoted with * ($p \leq 0.05$) and tendencies with + ($p \leq 0.1$). In the text box, p values for the main effect of treatment (TRT), time (T), and interaction of treatment and time (TRT × T) are shown [Color figure can be viewed at wileyonlinelibrary.com]

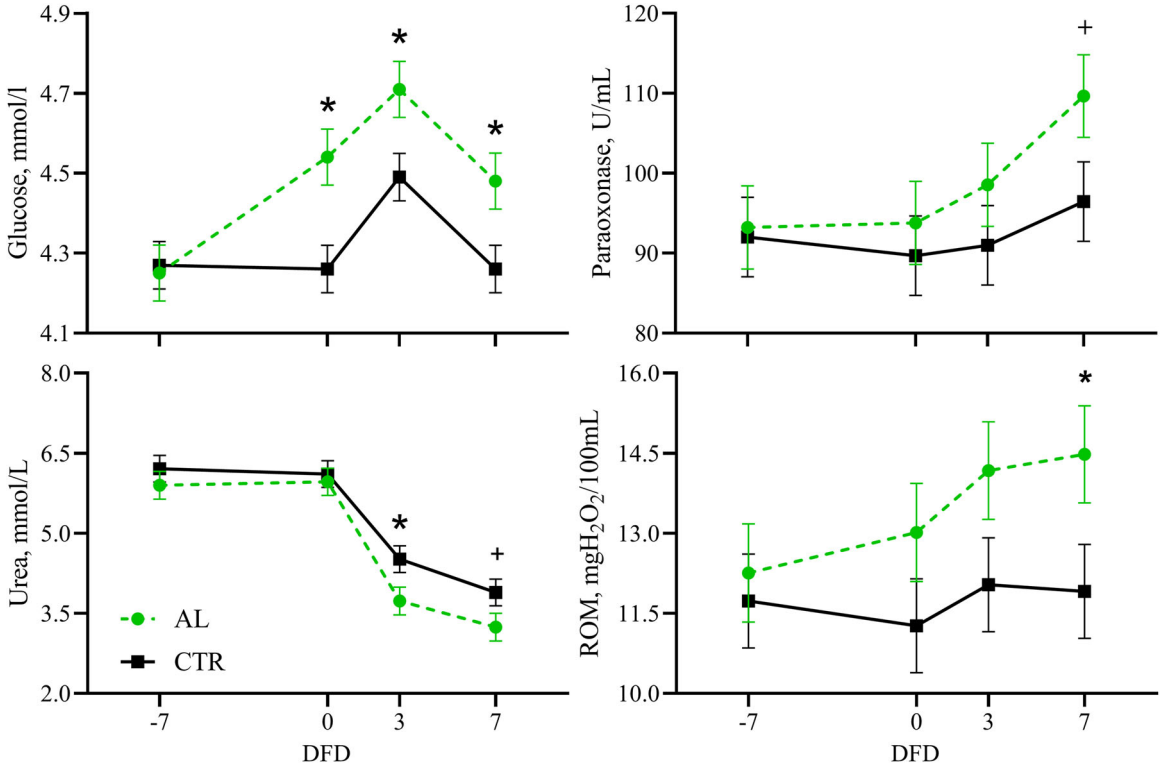

FIGURE 4 Plasma concentration of glucose, paraoxonase, urea, and reactive oxygen metabolites (ROM) from −7 to 7 days from dry-off (DFD) in dairy cows receiving 10 g/day of lyophilized *Aloe arborescens* Mill. (AL) from −7 to 7 days relative to dry-off or in the control group (CTR). Values presented as least squares means ± standard error. Differences between groups within each time point are denoted with * ($p \leq 0.05$) and tendencies with + ($p \leq 0.1$) [Color figure can be viewed at wileyonlinelibrary.com]

in vitro, whereas ours is indeed the first study evaluating rumen fermentations during *Aloe* spp. supplementation in vivo. Characterisation of ruminal microbial populations during aloe supplementation would help to explain the fermentation dynamic. Active compounds contained in *Aloe* might have caused a slowing of dry matter ruminal degradability, resulting in lower VFA production. However, our samples were collected once a day before the morning feeding when fermentation patterns reached their nadir. Therefore, differences in absorption or fermentation dynamic could have led to a discrepancy between actual VFA production and our measurements. Rumination time decreased immediately after dry-off resulting from the many different stressors occurring at this time (Abuelo et al., 2021), and

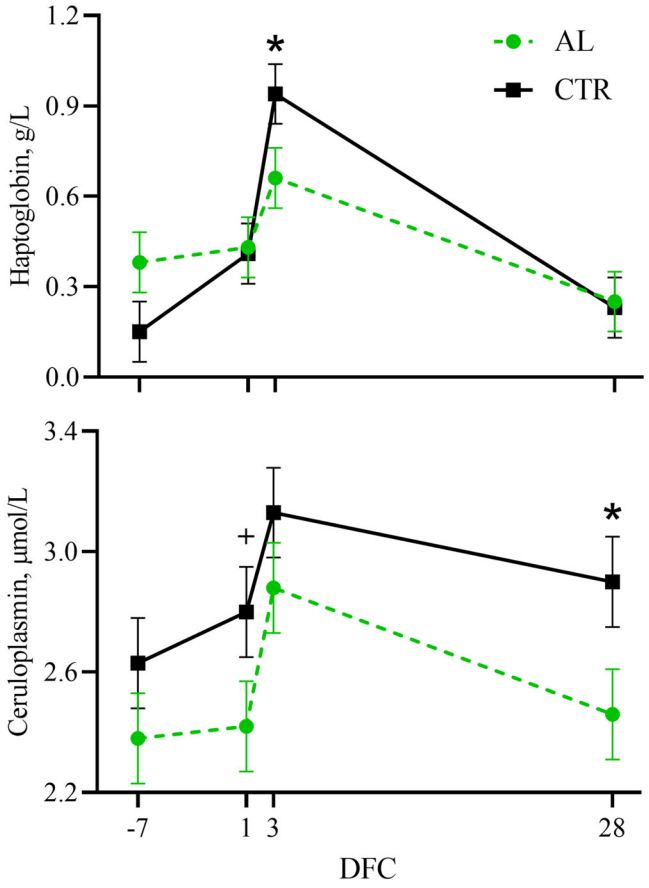

FIGURE 5 Plasma concentration of haptoglobin and ceruloplasmin from −7 to 28 days from calving (DFC) in dairy cows receiving 10 g/day of lyophilized *Aloe arborescens* Mill. (AL) from −7 to 7 days relative to dry-off or in the control group (CTR). Values presented as least squares means ± standard error. Differences between groups within each time point are denoted with * ($p \leq 0.05$) and tendencies with + ($p \leq 0.1$) [Color figure can be viewed at wileyonlinelibrary.com]

increased 1 week after despite the reduced dry matter intake, probably due to the adaptation to the diet of the dry period, higher in fiber than the lactation one. However, lyophilized *Aloe* supplementation did not significantly influence rumination time during the periods investigated. Despite the lack of differences at calving, the values observed in both groups were higher than those recorded by Calamari et al. (2014), suggesting a good metabolic condition in all enroled cows.

Cows supplemented with lyophilized *Aloe* had lower feces dry matter at dry-off and also at calving. Even though it is difficult to ascribe the lower dry fecal matter at calving to *Aloe* supplementation, the difference in this parameter at dry-off is noteworthy. *Aloe* spp. is also widely used by humans as a remedy for constipation (Cirillo & Capasso, 2015; Ramkumar & Rao, 2005), and its laxative effect has been confirmed in rats (Wintola et al., 2010). Aloin was indeed shown to cause an increase in water content and stimulation of peristalsis in rat intestines (Ishii et al., 1994). However, the reduction in dry matter did not result in issues, such as diarrhea, at the dosage of *Aloe* used in this study. Previous research has shown the lack of toxicity of *Aloe* whole leaf extract in mice and rats up to a dose of around 100 mg/kg/day (Guo & Mei, 2016; Matsuda et al., 2008). Therefore, the dose used in the present study, equivalent to approximately 15 mg/kg/day, was safe and should have not caused any toxic or adverse effects. Moreover, the metabolization of *Aloe* bioactive compounds that likely takes place in the bovine forestomaches further reduces the availability for gut absorption (Bani et al., 2016).

Aloe spp. is also known for its antimicrobial properties (Forno-Bell et al., 2019; Maan et al., 2018), and it has been used as an intramammary remedy to treat mastitis in organic systems (Pol & Ruegg, 2007). In the present study, we did not observe any effect of *Aloe* on SCC and mastitis incidence. *Aloe* was fed to dairy cows rather than applying it directly to the udder, and its main effects likely occurred in the digestive tract as previously described. Moreover, despite having not undergone antibiotic dry-cow therapy, cows were selected for good udder health at dry-off, and this selection might have limited the potential effects of *Aloe*. Furthermore, the presence of intramammary pathogens was similar between groups, both before dry-off and after calving. However, considering the limited number of subjects involved, the aim of these samplings was not to find differences in intramammary infection risk due to the oral supplement treatment but to identify potential factors that could have affected other results. Regardless of treatment, the lack of differences in mammary pathogen infections and SCC between dry-off and early lactation confirm the protective effects of internal teat sealant during the dry period when used alone without the antibiotic dry-cow therapy in healthy cows (Niemi et al., 2021; Winder et al., 2019), even when milk yield at dry-off is higher than the safety threshold proposed (Vilar & Rajala-Schultz, 2020).

Dramatic changes in plasma biomarkers happened around dry-off, as previously reported (Mezzetti et al., 2020b; Putman et al., 2018). In this scenario, the lyophilized *Aloe* administration caused a likely amelioration of liver function. These effects are similar to those reported by Mezzetti et al. (2020a) during the transition period. Aloin content was indeed lower in the lyophilized form we supplemented in the present study compared with the homogenate previously used. Nevertheless, *Aloe* contains a variety of bioactive compounds (e.g., phenols, flavonoids, vitamins), which have shown to have beneficial effects on human health and, together with aloin, could have been involved in the observed responses. Paraoxonase is an index of liver function (Bionaz et al., 2007). The higher concentration of paraoxonase suggested better liver function at the beginning of the dry period in the cows that received *Aloe*. Besides, the higher glycemia and the lower urea concentrations that resulted from *Aloe* supplementation might also be related to liver conditions. Glucose supply heavily relies on liver endogenous production, and impairment in liver function can harm glucose metabolism (Drackley et al., 2001; Hammon et al., 2009). Moreover, lower glucose levels can suggest an immune system activation at dry-off in the control group (Kvidera et al., 2017). Urea blood content is yielded by liver synthesis, starting from ammonia absorbed from the rumen (exogenous), or derived from deamination of amino acids (endogenous). In our experiment, since rumen ammonia was similar, the tendency towards lower urea

in AL cows could suggest a less recourse to deamination of amino acids in the liver, which, despite the slightly higher milk yield in AL and a similar rumination time between groups (i.e., a similar dry matte intake) could indicate a lower energy demand in AL cows, likely for a lower immunity system activity. In contrast, ROM concentrations increased in AL cows when compared with the CTR group for a few days starting at dry-off. *Aloe* is known to improve antioxidant availability (Ozsoy et al., 2009), even in cattle (Mezzetti et al., 2020a). During mammary gland involution, the production of oxidant species increases (Mezzetti et al., 2020b; Silanikove et al., 2005) in addition to the expression of several antioxidant genes (Singh et al., 2008). Excessive ROM concentration is suggestive of oxidative stress and inflammation (Celi & Gabai, 2015) although values recorded in AL were similar to those reported after dry-off in healthy cows in other studies (Cattaneo et al., 2021c; Mezzetti et al., 2020b; Putman et al., 2018). Moreover, the increase in ROM was paired with a general increase in the antioxidant capacity, as supported by the lack of differences in the OSI. Besides, ROM are markers of intense cell activity and are necessary for immune and inflammatory responses (Abuelo et al., 2015; Halliwell & Gutteridge, 2007; Kvietys & Granger, 2012). At the same time, paraoxonase, which is inversely related to oxidative stress (Turk et al., 2005), was slightly higher in AL cows. The slightly higher milk yield at dry-off that was observed in the AL group can hardly account for this difference. Another possible reason for this difference could be related to immune system activation. Among its many applications, *Aloe* spp. is used as drug absorption enhancing agent. *A. barbadensis* leaves have the potential to enhance drug permeation across the intestinal epithelial barrier thus causing an increase in tight junction permeability (Haasbroek et al., 2019). The possible increase in gastrointestinal permeability might have resulted in the translocation of lipopolysaccharides to the bloodstream (Gozho et al., 2005; Khafipour et al., 2009), causing the activation of the immune system and the consequent production of ROM (Bogdan et al., 2000). However, lipopolysaccharide translocation is known to induce a systemic inflammatory response (Ghosh et al., 2020; Minuti et al., 2014), and we did not observe that type of phenomenon in AL cows. Although the degree of this immune response can vary significantly (Plaizier et al., 2012), further research investigating the underlying mechanism of the ROM increase is needed.

The main result of *Aloe* administration on metabolites as reported by Mezzetti et al. (2020a) was an anti-hyperlipidemic effect. In that experiment, periparturient cows supplemented with *Aloe* showed an improvement in lipid metabolism, increase in BHB metabolization, and NEFA removal from the bloodstream, thus affecting milk fat output. In the current study, no effect was detected, and body fat mobilisation was not affected. However, the different phase during which *Aloe* was supplemented or the lower aloin content measured in the lyophilized form could account for this difference. The transition period is a period of intense lipid mobilisation, whereas, during the dry-off phase, this process is mild and the potential to reduce the NEFA concentration is limited.

Trying to connect changes in plasma biomarkers during the periparturient period to nutraceutical supplements given about 2 months earlier is difficult. However, as observed in previous studies, better conditions in the drying-off phase can influence the success of early lactation (Cattaneo et al., 2021a) or the development of early-lactation diseases (Abuelo et al., 2021). In the present study, lyophilized *Aloe* administration during dry-off seems to have led to a reduction in the magnitude of the acute phase liver response typical of the calving event (Trevisi & Minuti, 2018) as demonstrated by lower plasma haptoglobin and ceruloplasmin in this phase. The effect of *Aloe* might not have been direct but having improved cows' condition at dry-off could have ameliorated inflammatory status and metabolism during the dry period, allowing them to better adapt to the transition period. Haptoglobin and ceruloplasmin are indeed positive acute-phase protein concentrations (Ceciliani et al., 2012), and their plasma concentration increase because of this acute-phase response. Therefore, the better general condition observed both at dry-off and calving in the present study in AL cows could account for the slightly higher milk yield in the subsequent early lactation period. Our results seem to confirm previous observations suggesting that cows experiencing a better drying-off phase will also have an improved condition at lactation onset and produce more milk (Cattaneo et al., 2021a). Therefore, particular attention during the dry-off period is needed to ensure that cows are in proper health, metabolic, and inflammatory condition. Those cows will cope better with transition period challenges and likely have better performance during the subsequent lactation period.

5 | CONCLUSIONS

Our results indicate that supplementation of lyophilized *Aloe* in the dry-off period altered VFA proportions and lowered dry fecal content. Lyophilized *Aloe* also led to an improvement in liver function and increased the production of ROM. At the onset of lactation, cows that received *Aloe* at dry-off showed a decrease in inflammatory response after calving. Moreover, milk yield in the subsequent lactation was higher, whereas milk composition, SCC, and mastitis incidence were not affected by *Aloe* treatment. These results highlight the importance of improving cows' condition even before dry-off to obtain healthier cows during the transition period, particularly in cows not treated with antibiotics at milking cessation. Further research to elucidate *Aloe* spp. mode of action is needed.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Luca Cattaneo https://orcid.org/0000-0001-6027-7536

Fiorenzo Piccioli-Cappelli https://orcid.org/0000-0003-1277-7821

Andrea Minuti https://orcid.org/0000-0002-0617-6571

Erminio Trevisi https://orcid.org/0000-0003-1644-1911

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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