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RESEARCH ARTICLE

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Effects of dry-off or continuous lactation in Alpine and Saanen dairy goats carrying single or double kids on peripartum metabolic profile, performances, and milk composition

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ABSTRACT

Dairy goat's lactation persistence forces farmers at limiting nutrient supply to reduce yield at dry-off. Omitting dry period could be a solution, but metabolic effects of this practice have never been tested. Eight Alpine (AL) and 12 Saanen (SA) goats approaching their second kidding blocked by breed and number of kids carried (single - SIN - or double - DOU) were allocated to one out of two groups (4 AL and 6 SA; 5 SIN and 5 DOU in each group). At −42 ± 7 days from kidding (DFK), they were either dried off (DR) or milked continuously until kidding (CL). Body condition score (BCS) was assessed, and blood samples were collected at −10, −3, 5, 12, and 29 DFK to determine metabolic profile. Milk yield and composition were assessed at −56, 7, 31, 62, and 97 DFK. Compared with DR, CL had higher plasma reactive oxygen metabolites and liver enzymes. Compared with DR counterparts, AL-CL had higher nonesterified fatty acids (NEFA) at −10, whereas SA-CL had lower NEFA at −3 DFK. CL goats had lower BCS, higher plasma beta-hydroxybutyrate (BHB) and urea before kidding, but higher glucose at −3 and 5, lower NEFA at 5 and 12, and higher BCS at 29 DFK. CL goats had lower haptoglobin and myeloperoxidase at −3 and 5 DFK, paired with higher albumin, cholesterol, and paraoxonase at 12 DFK. Omitting dry period mitigated the inflammatory condition around kidding in dairy goats, possibly accounting for an improved energy balance in early lactation, despite body reserve mobilisation prepartum was greater under continuous lactation.

HIGHLIGHTS

- Omitting dry period accrued prepartum energy deficit in dairy goats but improved their post-partum energy balance.
- Omitting dry period mitigated acute phase response and inflammation in dairy goats during the late gestation and early lactation phases.
- Omitting dry period in high-yielding goats could allow for a smoother transition to the new lactation coping with the limitations hindering the dry-off procedures.

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Dairy goats; acute phase response; inflammation; energy metabolism; dry-off

Introduction

A two-months dry period represents the classical length for the non-lactating phase in all ruminant species. Several papers evaluated a shortened dry period as a potential strategy to increase milk produced in ruminants and improve the energy balance around delivery (Grummer and Rastani 2004). Outcomes obtained in dairy cows consistently documented that higher milk yields obtained by enlarging lactation length through shortening the dry phase (e.g. <30 days) were counterbalanced by a lower milk production in the subsequent lactation (Sørensen and Enevoldsen 1991; Watters et al. 2008; Kok et al. 2019), spanning between a 20 and 25% reduction when the dry period is completely omitted (Grummer and Rastani 2004; Pezeshki et al. 2010). This is due to the impairment of mammary renewal process when the dry phase is reduced (Oliver and Sordillo 1989). Alternatively, the mammary gland of dairy goats undergoes a continuous renewal

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process taking place during the whole lactation (Quintas et al. 2017). This physiological condition allows to omit the dry period without impairing mammary gland turnover or milk yield in the following lactation (Fowler et al. 1991; Caja et al. 2006).

The last two months of pregnancy represent the most challenging phase of the lactation cycle for dairy goats, as over 80% of kids weight is gained during this phase (Twardock et al. 1973; Lima et al. 2012a). Severe fluctuations affect the trend of steroid hormones (Liotta et al. 2021b) and protein and energy requirements of the dam increase dramatically, but feed intake is strongly limited by the volume of gravid uterus (Morand-Fehr 1989). The result is a negative energy balance, which induces a large mobilisation of body reserves and drives pregnant dairy goats to the edge of the physiological imbalance. The number of kids carried has a fundamental role in affecting the adaptation to the peripartum phase, as multiple pregnancies are accompanied by deeper metabolic changes and more severe hormonal fluctuation for the doe (Liotta et al. 2021a). Failure in the metabolic adaptation to late pregnancy could lead to a pathological condition, which involves abnormal ketone bodies release (Molina et al. 1991; Marutsova and Binev 2020), systemic inflammation, acute phase response and compromised liver function (D'Angelo et al. 2005; González et al. 2008; Djuricic et al. 2011; Janků et al. 2011). This condition is known as pregnancy toxaemia, and it has been described as the main reason for involuntary culling in small ruminants (Brozos et al. 2011; Lima et al. 2012b; Vasava et al. 2016). In this scenario, drying off high-yielding goats represents a challenge due to their great productive persistence, forcing farmers to rely on severe dietary restriction plans to achieve a 'classical' two-month dry period (Grummer and Rastani 2004; Cattaneo et al. 2023). For the most productive goats exceptional milk persistence at the end of lactation could necessitate a longer dietary restriction period, resulting in a shortened dry phase as compared to those of the other animals in the herd. Shortening the dry period while limiting nutrients supply potentially challenge dam's metabolism and kid's survival accruing prepartum energy deficit and inducing a failure in homeorhetic control mechanisms (Zobel et al. 2015), especially in goats carrying multiple kids. Nevertheless, omitting dry period has never been considered as a strategy to allow for a smoother metabolic adaptation to the late gestation and kidding phases. In dairy cows, alterations affecting the trends of plasma analytes have been widely used for monitoring the metabolic adaptation to the peripartum changes (Mezzetti et al. 2019; Cattaneo et al. 2021; Mezzetti and Trevisi 2023), but very little is known about the physiological trends of plasma markers in pregnant dairy goats.

Based on this background, this experiment aimed at 1) exploring peripartum trends of plasma analytes in Saanen and Alpine dairy goats carrying single or double kids, and 2) evaluating the effectiveness of omitting the dry period on improving the metabolic adaptation of the goats to the late gestation, kidding, and early lactation phases. Our hypothesis was that omitting dry period could improve the metabolic adaptation of dairy goats to the peripartum phase, especially when multiple kids are carried.

Materials and methods

This study complied with Italian laws on animal experimentation and ethics (DL n. 116, 27/01/1992) and all the experimental procedures were performed under the supervision of a veterinary practitioner. The trial was carried out on a commercial dairy farm ('Marta Emanuele', Arzago D'Adda, 24040, Bergamo, Italy), from December 2020 to June 2021.

Animal management and experimental design

A group of 28 primiparous dairy goats housed in a free stall pen was considered. While lactating, all the goats were milked twice daily at a 12-h interval (05:30 and 17:30 h) and received a diet formulated in accordance with National Research Council (1981) requirements, as reported in Table 1. A*d libitum* access to ryegrass hay was always guaranteed, 0.5 kg of alfalfa hay and 0.35 kg of concentrate were distributed after each milking, and 10 g of glycerol was top-dressed on the evening alfalfa hay meal. Two additional meals of 0.35 kg of concentrate each were distributed at 10:00 and 15:00 h and 0.10 kg of grounded corn was distributed at 2000 h. On 1st September, goats were seasonally synchronised applying the buck effect and they were artificially inseminated on their first visible natural heat within 15th September. Sixty days before the expected kidding (–56 ± 8 days from real kidding, **DFK**), goats were checked by echograph inspection to assess the number of kids carried and milk yield was recorded by dairy herd improvement check. Goats maintaining the greatest milk persistency at the end of lactation continued to receive the usual lactation diet and were milked continuously until kidding (**CL**; $n = 14$), while other goats underwent the dry-off routine (**DR**; $n = 14$). Briefly, DR goats were milked once

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

	Lactation	Dry
Diet		
Dry matter intake, kg	3.32	2.00
Ryegrass hay[a]	8.13	73.49
Alfalfa hay	26.81	–
Grass hay	27.41	–
Glycerol	0.31	0.01
Grounded corn	2.71	–
Concentrate	34.64	26.50
Concentrate composition		
Toasted soybean seed	5.90	
Pea meal	43.8	
Soybean hulls	8.34	
Corn gluten meal	8.00	
Wheat hulls	8.00	
Rice hulls	5.00	
Miller bran	5.00	
Saccharomyces cerevisiae	3.00	
Hydrogenated palm oil	6.30	
Maltodextrins	1.00	
Molasses	1.00	
Carob meal	0.50	
Calcium carbonate	2.00	
Calcium phosphate	0.20	
Sodium bicarbonate	0.50	
Magnesium oxide	0.10	
Sodium Chloride	1.00	
Supplement[b]	0.10	
Chemical composition		
UFL	0.89	0.72
Crude protein, % DM	17.8	13.1
Starch + sugar, % DM	17.9	14.6
Ether extract, % DM	4.4	3.5
NDF, % DM	40.2	58.1

[a]Offered ad libitum and estimated according to INRA equation.
[b]20000 UI of vitamin A, 8000 IU of vitamin D3, 100 mg of vitamin E, 0.39 mg of vitamin H1 (para-aminobenzoic acid), 100 mg of vitamin B1, 1.3 mg of vitamin B2, 94.9 mg of vitamin B3 (niacinamide), 1.5 mg of vitamin B6, 0.015 mg of vitamin B9 (folic acid), 0.013 mg of vitamin B12 (cyanocobalamin), 76.6 mg of choline, 8 mg of beta carotene, 58.7 mg of Mn, 109.8 mg of Zn, 14.3 mg of Cu, 2.1 mg of I, 0.045 mg of Co, 48.9 mg of Fe, 0.33 mg Se.
UFL: Unitè Fouraggère Lait; NDF: Neutral Detergent Fiber.

daily, and the amount of dietary concentrate was gradually decreased until complete suspension 47 days before the expected kidding (–42 ± 7 DFK) when the milk removal was halted. At −41 DFK, DR goats received the dry diet until kidding, consisting in *ad libitum* access to ryegrass hay, 3 meals of concentrate of 0.20 kg each between 07:30 h and 20:00 h, and 30 g of glycerol top-dressed on the hay at 2000 h (Table 1).

A subset of 20 pregnant dairy goats (10 CL and 10 DR), retrospectively selected to minimise milk yield differences among CL and DR groups, and blocked by breed and number of kids carried was enrolled in the study (4 Alpine –**AL**– and 6 Saanen –**SA**–; 5 single – **SIN** – and 5 double kidded goats – **DOU** –in each group).

Samples collection and animal measurements

Between −56 and 97 DFK, health status of the goats was monitored daily, and feeds were sampled at every change of the batches. Each kid was immediately weighed at kidding. At −56 ± 2.5 and then at 7 ± 2.5, 31 ± 8, 62 ± 8, and 97 ± 8 DFK, milk yield was recorded, and milk samples were collected by monthly dairy herd improvement checks in the morning and evening milking, alternatively. Furthermore, monthly data of dairy herd improvement checks performed during the first and the second lactation of the goats were used to calculate the total milk yield of the goats during the whole lactation. At −10 ± 2.6, −3 ± 1.2, 5 ± 1.8, 12 ± 2.0, and 29 ± 2.8 DFK, blood samples were collected before the morning feeding by jugular venipuncture into 10-mL evacuated heparinised tubes (BD Vacutainer, BD Diagnostics, Franklin Lakes, New Jersey, United States) and the body condition score (**BCS**) was evaluated by the same operator according to Villaquiran et al. (2007).

Analytical procedures

Feed samples were analysed for dry matter after oven drying at 65 °C until a constant weight was obtained. After grinding, samples were analysed for crude protein (992.23, AOAC, 2005), ash (942.05, AOAC, 2005), and starch (996.11, AOAC, 2005) using a K-TSTA assay kit (Megazyme International, Bray, Ireland). The neutral detergent fibre and nonfibrous carbohydrates were determined according to Van Soest et al. (1991).

Milk samples were analysed for fat, protein, and lactose contents by near-infrared instrumentation. The somatic cell count (**SCC**) was determined using an optofluorometric method with an automated cell counter (Fossomatic 180, Foss Analytics, Hillerød, Denmark) and expressed as a linear score (**LS**) according to Wiggans and Shook (1987).

Blood samples were processed and analysed in accordance with Calamari et al. (2016). A clinical autoanalyzer (ILAB-650, Instrumentation Laboratory, Lexington, Massachusetts, United States) was used to measure the concentration of glucose, nonesterified fatty acids (**NEFA**), beta-hydroxybutyrate (**BHB**), urea, creatinine, Ca, P, Mg, Na, K, Cl, Zn, haptoglobin, ceruloplasmin, total protein, albumin, globulin, cholesterol, aspartate aminotransferase (**AST-GOT**), gamma-glutamyl transferase (**GGT**) and alkaline phosphatase according to Calamari et al. (2016). Furthermore, paraoxonase, myeloperoxidase, and ferric reducing antioxidant power (**FRAP**) were determined as previously described (Minuti et al. 2015), thiol groups (**SHp**) according to Minuti et al. (2014) and total reactive oxygen metabolites (**ROMt**) according to Trevisi et al. (2015).

Statistical analysis

Data are presented as least squares means ± standard error (**SEM**). Data about milk yield and composition, BCS, and plasma analytes were submitted to ANOVA using a mixed model for repeated measures (GLIMMIX procedure, SAS Institute, Inc., Cary, North Carolina, United States). The fixed effects of group (**Gr**; CL, DR), breed (**Br**; AL and SA), kids number (**Kno**; SIN and DOU), and time, the first-order interactions of Gr x Br, Gr x Kno, Br x time, Kno x time and Gr x time, and the second-order interactions of Gr x Br x time and Gr x Kno x time were considered in the model. First and second-order interactions with time were dropped from the final model when no significant effect was detected. The second-order interaction of Gr x Br x Kno and the full interaction of Gr x Br x Kno x time were also tested but removed from the final model as no significant effect was detected in any of the explored parameters. The goat was considered as the random effect. Time had 5 levels for milk yield and composition (–56, 7, 31, 62, and 97 DFK) and 5 levels for plasma analytes (–10, −3, 5, 12, and 29 DFK).

The kids' weight and the milk yield over the first and second lactation of the dams were analysed by a one-way ANOVA (GLIMMIX procedure, SAS Institute, Inc., Cary, North Carolina, United States). For the milk yield over the first and second lactation, the model included the fixed effects of Gr, Br, and Kno, the first-order interactions of Gr x Br and Gr x Kno, and the goat was considered as a random effect. For kids' weight, the model also included the fixed effect of Sex (male or female). The first-order interaction of Gr x Sex was also tested but removed from the final model. The kid nested within the goat was considered the residual subject.

The analysis was carried out using 3 covariance structures and their heterogeneous counterparts, which were ranked according to their Akaike information criterion, with the one having the lowest Akaike information criterion being eventually chosen. The autoregressive covariance structure best fitted most of the parameters and was thus chosen for the analysis. The pair-wise comparison was done using the least significant difference test. The statistical significance and tendencies were declared at $p \leq 0.05$ and $p \leq 0.10$, respectively.

Results

All the explored parameters were affected by time, but time effect will not be presented in the results section and neither discussed thereafter.

Body condition score, kids' weight, milk yield, and milk composition

AL had lower BCS than SA goats and SIN tended to have higher BCS than DOU goats ($p = 0.01$ and 0.06, respectively; Table 2). Compared with DR, CL goats had a tendency towards lower BCS at −10 and had lower BCS at −3 DFK, but they had higher BCS at 29 DFK ($p < 0.1$, 0.05, and 0.05, respectively; Figure 1). Kids from AL goats had greater weight than those from SA goats and male kids had higher weight than female kids ($p = 0.03$ and 0.05, respectively; Table 2). Compared with DR, CL goats had higher milk yield at −56 DFK ($p < 0.05$; Figure 1). No effect was detected on the total milk yield calculated in the first and second lactation (Table 2).

Among milk components, DOU had higher milk protein content than SIN goats at 7 DFK ($p < 0.05$; Figure 2). Compared with DR, CL goats had lower butterfat at 7 and higher butterfat at 31 DFK ($p < 0.01$ and 0.05, respectively; Figure 1), higher protein ($p = 0.05$; Table 3), lower lactose at 31 and 62 DFK ($p < 0.01$ and 0.05, respectively; Figure 1) and a tendency towards higher SCC ($p = 0.09$; Table 2).

Plasma analytes

Energy, protein, and mineral metabolism

Among energy metabolism biomarkers, AL had higher NEFA than SA goats at −10 DFK ($p < 0.01$; Figure 3). Compared with DOU, SIN goats had lower NEFA at −3 DFK and lower BHB ($p < 0.01$ and 0.03; Figure 3 and Table 3). Compared with DR, CL goats had higher glucose at −3 and 5 DFK ($p < 0.05$ and < 0.01, respectively), higher NEFA at −10 ($p < 0.01$), but lower NEFA at 5 and 12 DFK ($p < 0.01$ and 0.05, respectively), a tendency towards higher BHB at −10 and higher BHB at −3 DFK ($p < 0.1$ and 0.05, respectively; Figure 4). Compared with DR counterparts, AL-CL goats had higher NEFA at −10 DFK ($p < 0.01$), but a tendency for lower NEFA at 5 DFK ($p < 0.1$), while SA-CL goats had lower NEFA at −3 and 5 DFK ($p < 0.01$ and 0.05, respectively, Figure 5).

Among protein metabolism biomarkers, AL had higher urea than SA goats ($p < 0.01$; Table 3). Compared with DR, CL goats had a tendency towards higher urea at −10 and had higher urea at −3 DFK ($p < 0.1$ and 0.01 respectively, Figure 4) and had lower creatinine ($p = 0.03$, Table 3). Compared with DR counterparts, SIN-CL goats had higher urea, while DOU-CL goats had lower creatinine ($p < 0.01$, 0.01; Table 3).

Among mineral metabolism biomarkers, AL tended to have higher Na at −10 and higher Na at 29 DFK

Table 2. Body condition score, kids' weight, milk yield, and milk composition in Alpine (AL) and Saanen (SA) dairy goats carrying single (SIN) or double kids (DOU) and milked continuously until kidding (CL) or dried-off at −56 days from kidding (DR).

Item[a]		Br		Kno				*p*-values[c]					
Unit	Gr	AL	SA	SIN	DOU		SEM[b]	Gr	Time	Br	Kno	Gr x Br	Gr x Kno
BCS	CL	2.30	2.58	2.46	2.42	**2.44**	0.11	0.43	<0.01	0.01	0.06	0.73	0.12
–	DR	2.35	2.69	2.70	2.34	**2.52**							
		2.32[a]	**2.64**[b]	**2.58**[a]	**2.38**[b]								
KBW[d]	CL	4.22	3.83	4.10	3.95	**4.02**	0.22	0.23	–	0.03	0.86	0.83	0.35
Kg	DR	4.03	3.55	3.68	3.90	**3.79**							
		4.12[a]	**3.69**[b]	**3.89**	**3.92**								
Milk yield	CL	4.81	4.90	5.00	4.71	**4.86**	0.37	0.58	0.02	0.86	0.67	0.90	0.62
kg/d	DR	4.62	4.64	4.61	4.64	**4.63**							
		4.71	**4.77**	**4.81**	**4.68**								
MYprev	CL	1477.3	1481.0	1538.4	1419.8	**1479.1**	116.9	0.11	–	0.55	0.84	0.55	0.36
Kg	DR	1241.2	1373.2	1269.7	1344.7	**1307.2**							
		1359.2	**1427.1**	**1404.1**	**1382.2**								
MYfoll	CL	938.5	1007.6	1069.4	876.7	**973.1**	86.8	0.85	–	0.36	0.48	0.95	0.16
Kg	DR	926.6	983.8	918.6	991.8	**934.2**							
		932.6	**995.7**	**994.0**	**934.2**								
Butterfat	CL	4.38	4.02	4.17	4.23[a]	**4.20**	0.28	0.50	<0.01	0.16	0.24	0.97	0.10
g/100g	DR	4.26	3.91	4.33	3.84[b]	**4.09**							
		4.32	**4.00**	**4.25**	**4.03**								
Protein	CL	3.79	3.67	3.67	3.79	**3.73**[a]	0.09	0.05	<0.01	0.87	0.64	0.23	0.36
g/100g	DR	3.51	3.60	3.57	3.54	**3.55**[b]							
		3.65	**3.63**	**3.62**	**3.66**								
Lactose	CL	4.42	4.45	4.43	4.43	**4.43**[a]	0.05	0.02	<0.01	0.75	0.98	0.88	0.96
g/100g	DR	4.55	4.56	4.56	4.56	**4.56**[b]							
		4.49	**4.50**	**4.49**	**4.50**								
SCC	CL	2.73	2.66	2.75	2.64	**2.69**[a]	0.10	0.09	0.01	0.97	0.58	0.43	0.53
Linear score	DR	2.50	2.57	2.53	2.54	**2.53**[b]							
		2.62	**2.61**	**2.64**	**2.59**								

[a]BCS is body condition score, KBW is kids birth weight; MYprev is milk yielded during the first lactation of the goats; MYfoll is milk yielded during the second lactation of the goats; SCC is somatic cell count.
[b]Standard error = largest standard error for the fixed effects.
[c]Gr is group effect (CL is continuous lactation, DR is dry period); Br is breed effect (AL is Alpine, SA is Saanen); Kno is kids number effect (SIN is single, DOU is double). Differences in each pairwise comparison are denoted with different superscripts (*ab* = $p < 0.1$; ab = $p < 0.05$).
[d]Sex was considered as additional fixed effect: $p = 0.05$.

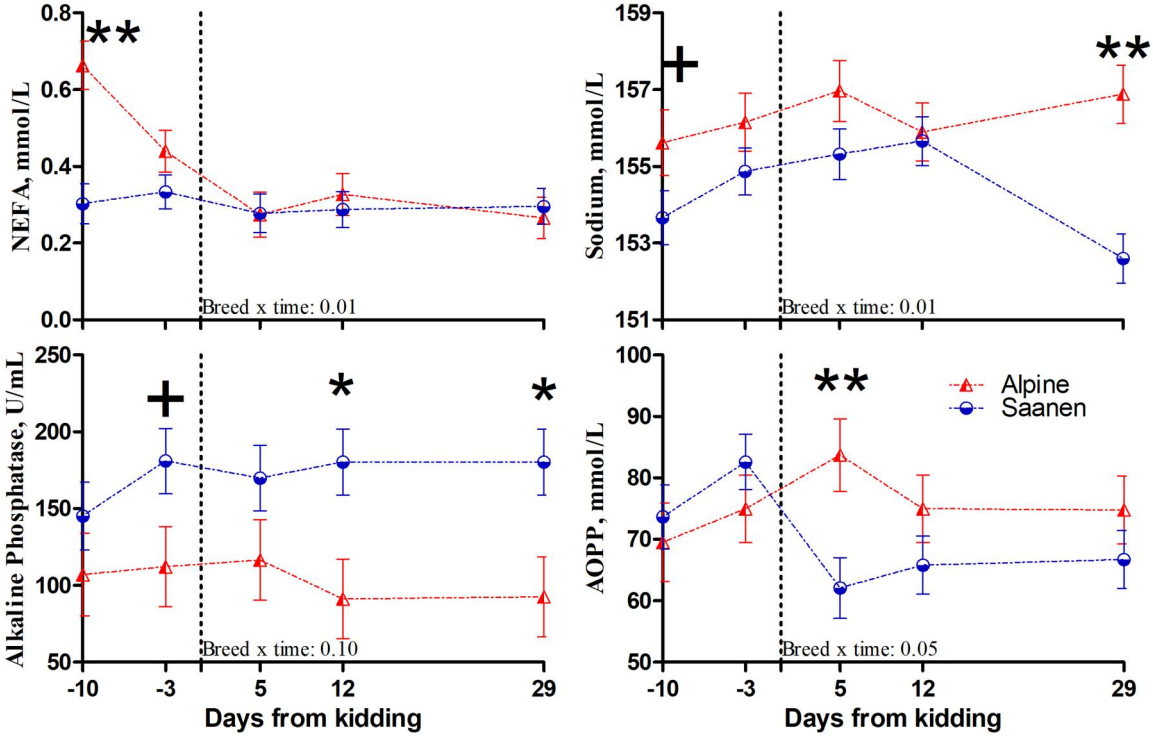

Figure 3. Patterns of plasma nonesterified fatty acids (NEFA), sodium, alkaline phosphatase, and advanced oxidation products of protein (AOPP) in Alpine and Saanen dairy goats. Differences at each time point are denoted with different symbols for the breed x time interaction (** is $p < 0.01$, * is $p < 0.05$, and + is $p < 0.1$).

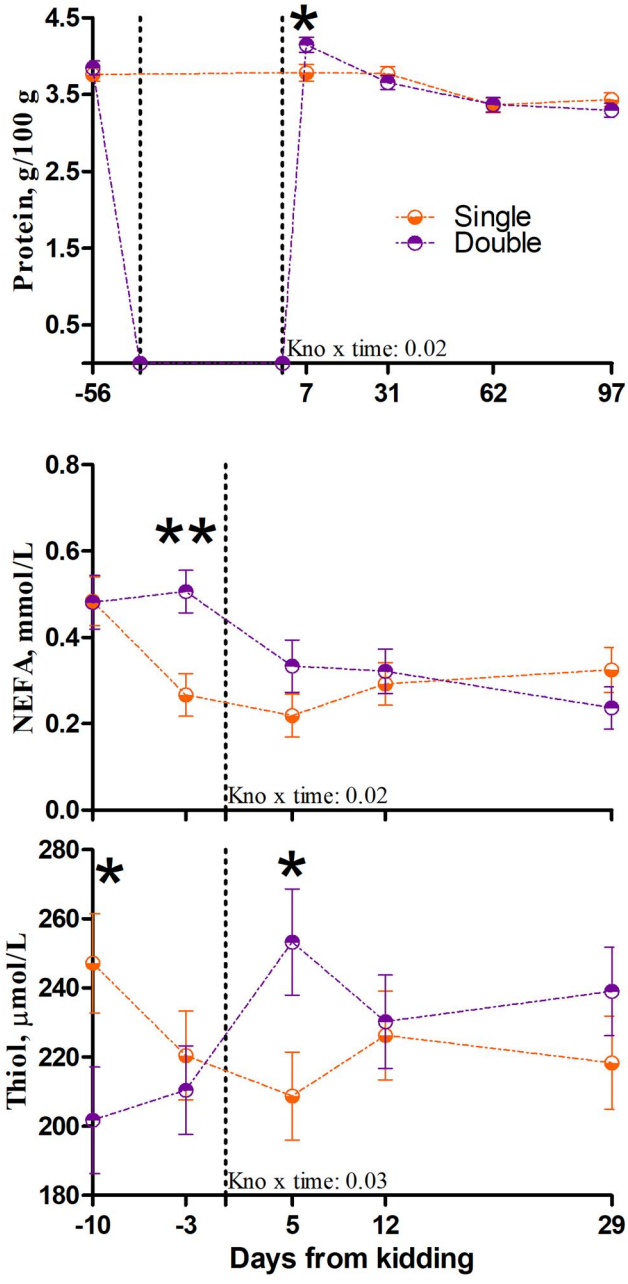

Figure 2. Patterns of milk protein content, plasma concentrations of nonesterified fatty acids (NEFA), and thiol groups in dairy goats carrying single or double kids. Differences at each time point are denoted with different symbols for the kids' number (Kno) x time interaction (** is $p < 0.01$ and * is $p < 0.05$).

($p < 0.1$ and 0.01, respectively; Figure 3) and higher Cl than SA goats ($p < 0.01$; Table 3). Compared with DOU, SIN goats tended to have lower Mg and higher Cl ($p = 0.09$ and 0.10, respectively; Table 3). Compared with DR, CL goats tended to have higher Ca and lower Cl and had lower P and Na ($p = 0.06$, 0.08, < 0.01, and < 0.01, respectively; Table 3). Compared with DR counterparts, AL-CL goats had higher Mg, and DOU-CL goats had lower Na ($p < 0.05$; Table 3). No effect was detected on K and Zn (Table 3).

Liver function and inflammation biomarkers

Among liver function biomarkers, AL had higher GOT ($p = 0.03$; Table 4), a tendency towards lower alkaline phosphatase at −10 and lower alkaline phosphatase at 12 and 29 DFK than SA goats ($p < 0.1$ and $p < 0.05$, respectively; Figure 3). Compared with DR, CL goats had higher GGT and alkaline phosphatase ($p = 0.05$ and 0.03, respectively; Table 4).

Among inflammation biomarkers, CL goats tended to have lower myeloperoxidase at −3 and lower myeloperoxidase at 5 DFK compared to DR goats ($p < 0.1$ and 0.01, respectively; Figure 4). No effect was noted in protein and globulin concentrations (Table 4). Among positive acute phase proteins, AL had higher ceruloplasmin than SA goats ($p = 0.05$; Table 4). Compared with DR, CL goats had lower haptoglobin ($p = 0.01$; Table 4). Among negative acute phase proteins, SIN had higher albumin and a tendency towards higher paraoxonase than DOU goats ($p = 0.01$ and 0.07, respectively; Table 4). Compared with DR, CL goats had higher albumin and cholesterol ($p = 0.03$ and 0.01, respectively; Table 4) and a tendency towards higher paraoxonase at 12 DFK ($p < 0.1$; Figure 4). Compared with DR counterparts, AL-CL goats had higher albumin ($p < 0.01$; Table 4).

Redox balance biomarkers

Among body antioxidant markers, AL had higher thiol groups than SA goats ($p < 0.01$; Table 4). Compared with DOU, SIN goats had higher thiol groups at −10 and lower thiol groups at 5 DFK ($p < 0.05$; Figure 2). Among oxidant species, CL goats tended to have higher ROMt than DR goats ($p = 0.07$; Table 4). Among oxidative stress products, AL goats had higher AOPP at 5 DFK compared to SA goats (Figure 1).

Discussion

The trends of BCS and plasma analytes reflecting energy and protein metabolism detected here (i.e. the peaks in NEFA and BHB and the drops in glucose and urea) confirm that late pregnancy is the phase inducing the greatest mobilisation of body reserves across the whole peripartum period in dairy goats. Conversely, the trends of liver function, inflammation, and redox balance biomarkers seem to point to a systemic inflammatory condition and an acute phase response occurring around kidding, mimicking those

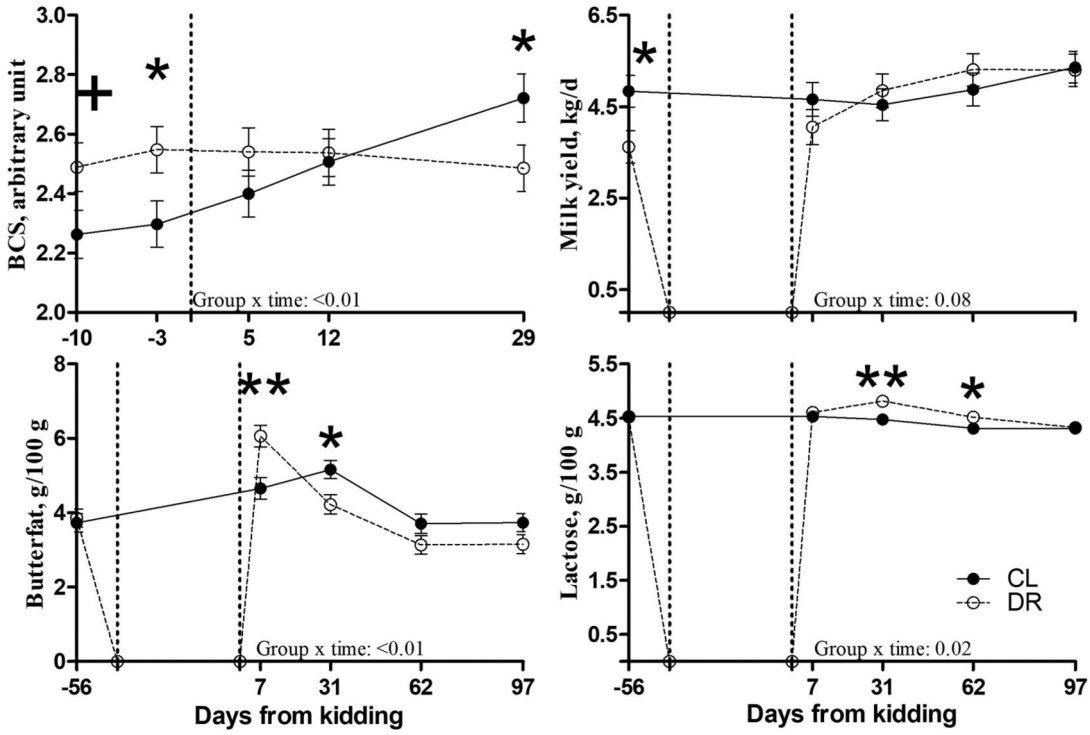

Figure 1. Patterns of body condition score (BCS), milk yield, butterfat, and lactose in dairy goats milked continuously until kidding (CL) or dried off at −56 days from kidding (DR). Differences at each time point are denoted with different symbols for the group x time interaction (** is $p < 0.01$, * is $p < 0.05$, and + is $p < 0.1$).

documented in peripartum dairy cows. The effect of breed and number of kids carried on the trends of these biomarkers have been scarcely investigated in dairy goats and, to the best of our knowledge, this is the first study exploring the effect of omitting dry period on the physiological processes taking place in this phase. In field conditions, goats selected for continuous lactation within a herd are those maintaining the greatest milk persistence at the end of lactation. Despite we selected a small number of goats to obtain homogeneous groups, the higher milk yield that CL goats had at −56 DFK should be considered as a limitation in our study. Furthermore, as a field study, our experiment was not designed to provide a punctual evaluation of the individual feed intake or daily milk yield of the goats. The lack of any individual monitoring system forced us to rely on data provided by monthly dairy herd tests for milk yield and composition and did not allow us to collect any data on the feed intake of the goats. Finally, quality of colostrum was not evaluated, despite this is a fundamental aspect to consider for implementing the omission of the dry phase in commercial dairy herds. Thus, the results obtained here should be considered as preliminary data and confirmed in larger experiments where homogeneous experimental group are enrolled, individual feed intake and milk yield of the goats are measured daily and colostrum samples are collected.

Adaptation to the transition period is differentially affected by breed and number of kids carried

Carrying multiple kids have been widely documented as a primary cause for altered body homeostasis in goats approaching kidding (Conway et al. 1996; Celi et al. 2008; Cappai et al. 2019). In this study, this is supported by the lower BCS and the higher plasma NEFA and BHB concentrations found in DOU compared with SIN goats, suggesting that the demand of multiple kids boosted body fat mobilisation and ketone body release before parturition. Furthermore, the lower albumin and paraoxonase reflect a more severe acute phase response in DOU compared with SIN goats. Both analytes are known as negative acute phase proteins (Ceciliani et al. 2012), and confirm the massive metabolic load driven by multiple kids as an acute phase response inductor (D'Angelo et al. 2005; Juárez-Reyes et al. 2008; Anwar et al. 2012; Castagnino et al. 2015).

Table 3. Plasma biomarkers of energy, protein, and mineral metabolism in Alpine (AL) and Saanen (SA) dairy goats carrying single (SIN) or double kids (DOU) and milked continuously until kidding (CL) or dried-off at −56 days from kidding (DR).

Item[a]	Gr	Br		Kno				*p*-value[c]					
Unit		AL	SA	SIN	DOU		SEM[b]	Gr	Time	Br	Kno	Gr x Br	Gr x Kno
Glucose	CL	3.92	4.09	3.95	4.07	**4.01**^{A}	0.12	0.01	0.07	0.44	0.41	0.42	0.79
mmol/L	DR	3.70	3.70	3.67	3.73	**3.70**^{B}							
		3.81	**3.89**	**3.81**	**3.90**								
NEFA	CL	0.43	0.22^{A}	0.31	0.34	**0.32**	0.03	0.29	<0.01	0.03	0.16	0.01	0.54
mmol/L	DR	0.36	0.38^{B}	0.33	0.41	**0.37**							
		0.39^{A}	**0.30**^{B}	**0.32**	**0.38**								
BHB	CL	0.60	0.46	0.50	0.56	**0.53**	0.04	0.69	0.21	0.23	0.03	0.14	0.24
mmol/L	DR	0.50	0.52	0.42	0.60	**0.51**							
		0.55	**0.49**	**0.46**^{A}	**0.58**^{B}								
Urea	CL	10.45	8.63	9.60^{A}	9.49	**9.54**^{a}	0.27	0.08	<0.01	<0.01	0.11	0.23	0.06
mmol/L	DR	9.37	8.42	8.26^{B}	9.53	**8.89**^{b}							
		9.91^{A}	**8.53**^{B}	**8.93**	**9.51**								
Creatinine	CL	71.6	70.6	73.9	68.3^{A}	**71.1**^{a}	2.00	0.03	<0.01	0.35	0.91	0.20	0.06
µmol/L	DR	74.6	80.6	75.1	80.1^{B}	**77.6**^{b}							
		73.1	**75.6**	**74.5**	**74.2**								
Ca	CL	2.53	2.58	2.59	2.52	**2.56**^{a}	0.04	0.06	0.01	0.56	0.35	0.65	0.62
mmol/L	DR	2.45	2.46	2.47	2.44	**2.45**^{b}							
		2.49	**2.52**	**2.53**	**2.48**								
P	CL	1.89	1.89	2.00	1.78	**1.89**^{A}	0.14	<0.01	0.68	0.29	0.80	0.29	0.36
mmol/L	DR	2.70	2.30	2.44	2.56	**2.50**^{B}							
		2.29	**2.09**	**2.22**	**2.17**								
Mg	CL	1.17^{a}	1.08	1.09	1.15	**1.12**	0.03	0.15	0.39	0.81	0.09	0.03	0.95
mmol/L	DR	1.04^{b}	1.11	1.04	1.10	**1.07**							
		1.10	**1.09**	**1.07**^{a}	**1.13**^{b}								
Na	CL	155.2	152.9	154.3	153.8^{a}	**154.1**^{A}	0.50	<0.01	0.12	0.01	0.30	0.53	0.06
mmol/L	DR	157.4	155.9	155.7	157.6^{b}	**156.7**^{B}							
		156.3^{A}	**154.4**^{B}	**155.0**	**155.7**								
K	CL	4.60	4.62	4.60	4.62	**4.61**	0.09	0.44	<0.01	0.99	0.78	0.88	0.66
mmol/L	DR	4.53	4.51	4.56	4.47	**4.52**							
		4.57	**4.56**	**4.58**	**4.55**								
Cl	CL	115.1	111.7	113.7	113.1	**113.4**^{a}	0.50	0.08	<0.01	<0.01	0.10	0.65	0.40
mmol/L	DR	116.6	112.6	115.4	113.8	**114.6**^{b}							
		115.8^{A}	**112.2**^{B}	**114.5**^{a}	**113.4**^{b}								
Zn	CL	9.17	10.11	10.17	9.12	**9.64**	0.76	0.62	0.67	0.53	0.45	0.77	0.76
µmol/L	DR	8.98	9.31	9.36	8.92	**9.14**							
		9.08	**9.71**	**9.76**	**9.02**								

[a]NEFA is nonesterified fatty acids, BHB is beta-hydroxybutyrate, Ca is calcium, P is phosphorus, Mg is magnesium, Na is sodium, K is potassium, Cl is chlorine, and Zn is zinc.
[b]Standard error = largest standard error for the fixed effects.
[c]Gr is group effect (CL is continuous lactation, DR is dry period); Br is breed effect (AL is Alpine, SA is Saanen); Kno is kids number effect (SIN is single, DOU is double). Differences in each pairwise comparison are denoted with different superscripts (*ab* = $p < 0.1$; ab = $p < 0.05$; AB = $p < 0.01$).

Less clear and defined is the different metabolic adaptation to the peripartum phase occurring in the two different breeds raised under the same management practices. The higher BCS found in SA goats and their lower plasma NEFA concentration suggest that this breed faced a milder mobilisation of body fat compared with AL (Dunshea et al. 1989). This is consistent with the lower plasma urea detected in SA goats, reflecting a lower amount of amino acids mobilised from muscle tissue and deserved to deamination processes to face the energy deficit (Laporte-Broux et al. 2011; Madan et al. 2020). Thus, the higher birthweight of AL kids compared with SA could have been the result of a greater diversion of dam's energy and protein sources to foetal growth. Despite the limitations hindering our experiment do not allow us to exclude that the different energy status between the two breeds depended on a different feed intake, trends of other plasma analytes suggest that a different metabolic adaptation to the peripartum changes possibly accounted for it. Indeed, the higher plasma concentration of alkaline phosphatase suggests greater liver activity in SA goats, and the lowered plasma concentrations of Na and Cl suggest that this breed could have faster blood circulation compared with AL, as these electrolytes are strongly bond to fluid distribution (Gipson and Grossman 1990; Cappai et al. 2019). Thus, the better energy status of SA goats could depend on a more efficient metabolism of this breed. Conversely, the higher plasma ceruloplasmin reflects a greater acute phase condition occurring in AL goats (Ceciliani et al. 2012). This could account for the elevated plasma AST-GOT concentration detected in this breed, as acute phase response has been widely listed as a cause of liver damage (Trevisi et al. 2012; Rodriguez-Jimenez et al. 2018; Mezzetti et al. 2020a). Leucocyte activation accompanying acute phase response could also account for the higher

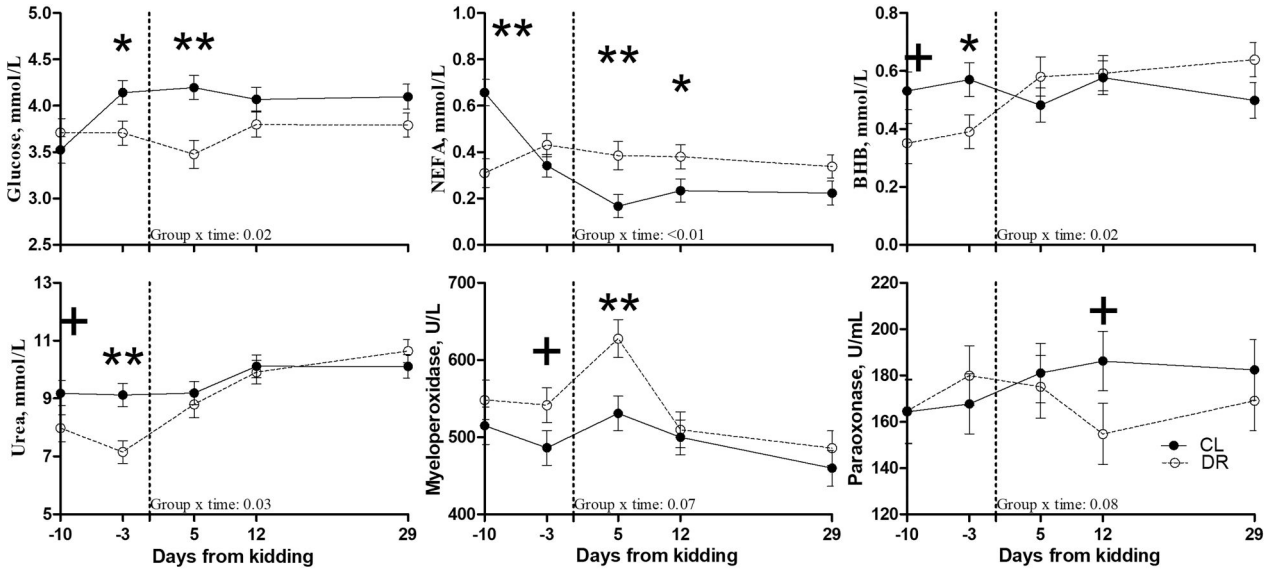

Figure 4. Patterns of plasma concentration of glucose, nonesterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), urea, myeloperoxidase, and paraoxonase in dairy goats milked continuously until kidding (CL) or dried off at −56 days from kidding (DR). Differences at each time point are denoted with different symbols for the group x time interaction (** is $p < 0.01$, * is $p < 0.05$, and + is $p < 0.1$).

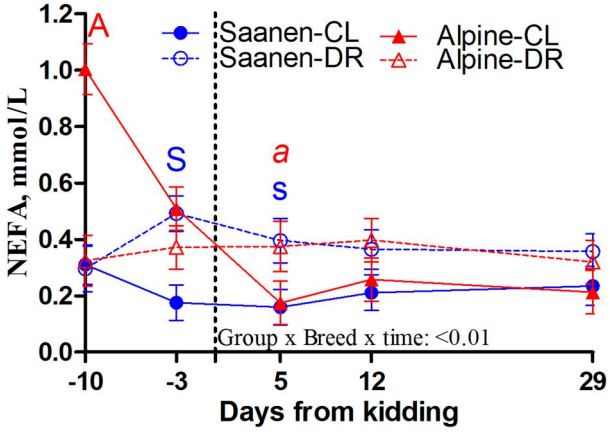

Figure 5. Patterns of plasma concentration of nonesterified fatty acids (NEFA) in Alpine and Saanen dairy goats milked continuously until kidding (CL) or dried off at −56 days from kidding (DR). Differences at each time point are denoted with different letters for the group x breed x time interaction ('S' is $p < 0.01$ and 's' is $p < 0.05$ for Saanen goats; 'A' is $p < 0.01$, '*a*' is $p < 0.10$ for Alpine goats).

endogenous antioxidants (i.e. thiol groups) and AOPP concentration detected in AL goats. In fact, heightened release of oxidant species by leukocytes upregulates endogenous antioxidant syntheses, and AOPP serves as a marker of protein oxidation driven by leukocyte metabolites (Celi and Gabai 2015). In this context, inflammatory and acute phase conditions could account for the greater energy deficit experienced by AL goats. Liver damage induced by acute phase response impairs NEFA oxidation and promotes triglycerides accumulation in the liver through inhibiting the export of mobilised NEFA *via* very low density lipoproteins (Kleppe et al. 1988; Bertoni et al. 2006; Janovick et al. 2022). Lowered gluconeogenetic efficiency due to impaired liver function (Loor et al. 2007) paired with heightened glucose consumption by activated leukocytes (Kvidera et al. 2017) has both been listed as predisposing factors for developing pregnancy toxaemia (González et al. 2011) and ketosis condition (Mezzetti et al. 2019).

Omitting dry period positively affected the adaptation to the new lactation by mitigating the inflammatory state

All the CL goats considered in this study had an average daily yield ≥ 2.5 L/d during the last two month of gestation, and milk secretion was continuously maintained till kidding. Under continuous lactation, the greater Ca sequestration by the mammary gland possibly induced a faster mobilisation of this mineral from the bones (Horst et al. 1997), accounting for the higher circulating Ca of CL goats. Furthermore, the faster fluid redistribution driven by milk synthesis is consistent with the lowered plasma concentration of P (Cappai et al. 2019). Moreover, the longer oxidative metabolism taking place in the mammary gland during galactopoietic processes could account for the higher plasma ROMt concentration in CL goats (Celi and Gabai 2015). Maintaining milk synthesis until kidding likely boosted the prepartum energy deficit and induced a greater mobilisation of body reserves before

Table 4. Plasma biomarkers of liver function, inflammation, and redox balance in Alpine (AL) and Saanen (SA) dairy goats carrying single (SIN) or double kids (DOU) and milked continuously until kidding (CL) or dried-off at −56 days from kidding (DR).

Item[a]	Gr	Br		Kno			SEM[b]	*p*-value[c]					
Unit		AL	SA	SIN	DOU			Gr	Time	Br	Kno	Gr x Br	Gr x Kno
GOT	CL	122.1	116.3	113.5	124.8	**119.2**	6.9	0.42	0.07	0.03	0.37	0.10	0.71
U/L	DR	144.9	108.1	124.2	128.8	**126.5**							
		133.5[a]	**112.2**[b]	**118.9**	**126.8**								
GGT	CL	50.6	56.6	48	59.2	**53.6**[a]	4.7	0.05	<0.01	0.36	0.49	0.96	0.25
U/L	DR	38.3	43.7	42.5	39.5	**41.0**[b]							
		44.4	**50.1**	**45.2**	**49.4**								
ALP	CL	131.8	216.7	193.3	155.1	**174.2**[a]	23.8	0.03	0.22	0.04	0.45	0.57	0.61
U/L	DR	76.2	126.1	105	97.3	**101.2**[b]							
		104.0[a]	**171.4**[b]	**149.2**	**126.2**								
Protein	CL	78.3	76.6	78.7	76.2	**77.4**	1.2	0.89	0.02	0.72	0.16	0.15	0.79
g/L	DR	75.8	78.6	78.1	76.3	**77.2**							
		77	**77.6**	**78.4**	**76.2**								
Globulin	CL	38.4	39.7	39.3	38.9	**39.1**	1.2	0.33	0.03	0.36	0.76	0.96	0.95
g/L	DR	39.8	41.3	40.9	40.3	**40.6**							
		39.1	**40.5**	**40.1**	**39.6**								
Myeloperoxidase	CL	521.6	475.1	500.1	496.6	**498.3**[a]	17.8	0.07	<0.01	0.16	0.88	0.59	1.00
U/L	DR	553.2	531.9	544.4	540.7	**542.6**[b]							
		537.4	**503.5**	**522.2**	**518.7**								
Haptoglobin	CL	0.16	0.26	0.16	0.26	**0.21**[a]	0.05	0.01	<0.01	0.67	0.12	0.35	0.80
g/L	DR	0.41	0.37	0.33	0.45	**0.39**[b]							
		0.28	**0.31**	**0.24**	**0.35**								
Ceruloplasmin	CL	4.38	3.94	4.38	3.93	**4.16**	0.18	0.28	<0.01	0.05	0.40	0.85	0.28
µmol/L	DR	4.17	3.64	3.87	3.93	**3.9**							
		4.28[a]	**3.79**[b]	**4.13**	**3.93**								
Albumin	CL	39.7[A]	36.9	39.5	37.1	**38.3**[a]	0.5	0.03	0.05	0.24	0.01	0.01	0.39
g/L	DR	36.1[B]	37.3	37.3	36.1	**36.7**[b]							
		37.9	**37.1**	**38.4**[A]	**36.6**[B]								
Cholesterol	CL	4.83	4.36	4.81	4.38	**4.59**[A]	0.25	0.01	0.10	0.20	0.20	0.91	0.98
mmol/L	DR	3.88	3.49	3.89	3.48	**3.69**[B]							
		4.35	**3.92**	**4.35**	**3.93**								
Paraoxonase	CL	162.7	189.9	178.4	174.3	**176.3**	11.8	0.62	0.40	0.35	0.07	0.41	0.12
U/mL	DR	167.7	169.6	194.9	142.4	**168.7**							
		165.2	**179.8**	**186.7**[a]	**158.3**[b]								
SHp	CL	232.8	214.7	225.3	222.2	**223.7**	7.6	0.71	0.75	<0.01	0.78	0.17	0.56
mmol/L	DR	250.4	204.5	223.2	231.8	**227.5**							
		241.6[A]	**209.6**[B]	**224.2**	**227**								
ROMt	CL	22.2	20.4	22.1	20.5	**21.3**[a]	1.1	0.07	<0.01	0.18	0.31	0.82	0.68
mg H_2O_2/100 mL	DR	19.8	18.6	19.5	18.8	**19.2**[b]							
		21	**19.5**	**20.8**	**19.7**								
AOPP	CL	75.2	75.7	76.1	74.8	**75.5**	2.9	0.19	0.44	0.17	0.54	0.14	0.79
mmol/L	DR	76	64.7	72	68.6	**70.3**							
		75.6	**70.2**	**74.1**	**71.7**								

[a]GOT: glutamate-oxalacetate transaminase, GGT: γ-glutamyl transferase, ALP: alkaline phosphatase, SHp: thiol groups, ROMt : total reactive oxygen metabolites, and AOPP: advanced oxidation product of protein.
[b]Standard error: largest standard error for the fixed effects.
[c]Gr : group effect (CL is continuous lactation, DR is dry period); Br : breed effect (AL is Alpine, SA is Saanen); Kno: kids number effect (SIN : single, DOU is double). Differences in each pairwise comparison are denoted with different superscripts (*ab* = $p < 0.1$; ab = $p < 0.05$; AB = $p < 0.01$).

kidding. This is consistent with the lower BCS and higher plasma BHB and urea that CL goats had in this phase. It could also account for the lower plasma creatinine observed in CL goats. The latter reflects a lower consumption of phosphocreatine by the muscles (Hayden et al. 1992; Osorio et al. 2014), suggesting that CL goats had a lower muscle mass compared with DR goats and confirming the greater diversion of body protein to gluconeogenesis suggested by urea trends. A different response to continuous lactation was detected on prepartum tends of plasma NEFA in the two breeds considered, as AL-CL goats had higher NEFA whereas SA-CL had lower NEFA compared with their DR counterparts. This could have been driven by the different nutrient status of the two breeds, possibly penalising AL goats when prepartum energy deficit was accrued by maintaining milk production. Greater prepartum mobilisation of body reserves in CL goats is consistent with their higher plasma GGT and alkaline phosphatase concentrations, reflecting a greater liver load to oxidise mobilised NEFA (Rodriguez-Jimenez et al. 2018).

Nevertheless, a greater glucose concentration was consistently detected in CL goats in late gestation, and no difference was detected on the birthweight of kids of CL compared to those of DR goats. The latter suggests that omitting dry period improved liver efficiency in utilising mobilised NEFA (Radin et al. 2015; Zamuner

et al. 2020) and this, even combined with the greater energy and protein intake ensured by the lactation diet administered in this study, allowed the maintaining of milk synthesis without impairing the growth of kids during the late gestation phase. This could have also ameliorated the energy balance postpartum, as suggested by the higher glucose and lower NEFA found in plasma of CL goats during the first two weeks after kidding, and by the higher BCS they had at 29 DFK. Ameliorated postpartum energy balance is also consistent with higher protein and lower butterfat found in the milk of CL goats during the first week after kidding, as milk protein synthesis directly depends on available energy (Doepel et al. 2004), and blood NEFA contributes to milk fat content when severe lipid mobilisation occurs (Jorjong et al. 2014).

Regarding milk composition and performances in the following lactation, the tendency towards higher SCC found in CL goats suggests that continuous lactation possibly affected mammary gland health. This is further supported by the lowered Na and Cl found in plasma and by the lowered lactose found in milk of CL goats at the onset of lactation. In dairy cows, leukocytes activities taking place in the mammary gland increase the permeability of the epithelial tissue, leading to a greater influx of electrolytes and a greater efflux of lactose from the udder to the blood (Oliver and Calvinho, 1995; Aslam and Tucker, 1998). However, these physiological alterations have been scarcely investigated in dairy goats with compromised udder health, and the lack of any determination of the electrolyte concentration in milk of our CL goats does not allow us to confirm this interpretation. Furthermore, the lack of any effect on milk yield both in early and the whole lactation dismisses any negative effect of omitting dry period on productive performances of the goats, in accordance with previous studies (Fowler et al. 1991).

The lower concentration of haptoglobin, the higher concentration of paraoxonase at 12 DFK, and the higher concentrations of albumin and cholesterol (reflecting that of circulating lipoproteins) observed throughout the experimental period pointed to a reduced acute phase response in CL goats (Bertoni et al. 2008; Ceciliani et al. 2012). Particularly, AL goats had higher plasma albumin concentration under continuous lactation compared with their DR counterparts. The lower acute phase response degree in CL goats is consistent with their lower concentration of plasma myeloperoxidase, reflecting a milder neutrophil activation around the delivery day (Faith et al. 2008). Altogether, beneficial effects sorted by omitting dry period on mitigating acute phase response and inflammatory conditions could account for the improved energy metabolism of CL goats at the onset of lactation. The mechanism driving the mitigation of the inflammatory conditions experienced by CL goats around kidding is not clear. In dairy cows, dry-off procedures induces a systemic inflammatory state accompanying early stages of mammary gland involution (Putman et al. 2018; Mezzetti et al. 2020b), and the severity of such a condition has been related to those of the acute phase response occurring in early lactation (Cattaneo et al. 2021; Mezzetti et al. 2021). Despite that, our experiment was not designed to detect inflammatory conditions induced by milking interruption in our DR goats, and future research on this topic should collect blood samples around dry-off (i.e. the day before and three days after milking interruption) to fully elucidate the effect of this practice on plasma analyte trends.

Conclusions

Outcomes obtained in this study confirmed carrying multiple kids as a stressor for dairy goats, boosting body fat mobilisation and inducing an acute phase response. Furthermore, the different trends detected in energy, protein, and mineral metabolism, and inflammation biomarkers suggest that SA and AL goats have different adaptation to this challenging phase. Omitting dry period improved the adaptation of the goats to the peripartum phase mitigating acute phase response and ameliorating the energy balance at the onset of lactation, irrespectively of breed and number of kids carried. Based on these outcomes, omitting dry period while applying adequate feeding and management practices could be considered an effective strategy for improving the adaptation of dairy goats to the peripartum phase. Nevertheless, this should be considered as a pilot study and opens new questions requiring further investigations. Milk and colostrum composition should be investigated deeply, and the long-term effect on animal welfare and performance should be evaluated carefully before considering repeated omission of the dry period as a recommended management practice in dairy goats' herds.

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Ethical approval

This study complied with Italian laws on animal experimentation and ethics (DL n. 116, 27/01/1992) and all the experimental procedures were performed under the supervision of a veterinary practitioner.

Disclosure statement

The authors declare that there is no conflict of interest associated with the paper. The authors alone are responsible for the content and writing of this article.

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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.

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