



AKADÉMIAI KIADÓ

# Effect of postpartum drenching on plasma parameters of cows at a large-scale dairy farm

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## RESEARCH ARTICLE



## ABSTRACT

The aim of the present study was to explore the influence of postpartum drenching with a feed additive on the plasma concentration of biochemical parameters while factoring in prepartum rumination times (RT). One hundred and sixty-one cows were fitted with a Ruminact® HR-Tag approximately 5 days before calving. Drenching and control groups were established based on calving dates. Animals in the drenched group were treated three times (Day 1/day of calving/, Day 2, and Day 3 postpartum) using a feed additive containing calcium propionate, magnesium sulphate, yeast, potassium chloride and sodium chloride mixed in approximately 25 L of lukewarm tap water. Blood samples were collected on Days 1, 2, 3, 7 and 12. Cows with below the average RT were categorised as “low rumination” and those above it as “high rumination” animals. Drenching decreased the plasma concentrations of total protein, urea and creatinine and increased the levels of alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT) and chloride. Low rumination time prepartum resulted in higher concentrations of beta-hydroxybutyrate, total protein and activities of alkaline phosphatase and GGT, while it decreased the activity of ALT and the concentrations of calcium, magnesium, sodium and potassium. The day of lactation had an effect on all parameters except for potassium.

## KEYWORDS

dairy cattle, rumination time, BHB, NEFA, calcium

## INTRODUCTION

The transition period, from three weeks prepartum to three weeks postpartum, is the most challenging time for a dairy cow (Grummer, 1995). During this time, there is a marked decrease in dry matter intake (Bertics et al., 1992), which, together with the onset of the lactation, causes a negative energy balance (NEB) (Drackley et al., 2001). This leads to an increased mobilization of non-esterified fatty acids (NEFA) from the adipose tissue to the liver, where NEFA exhausts its oxidation capacity and contributes to liver associated diseases like ketosis or fatty liver disease (Ringseis et al., 2014). Disturbed ruminal function around the time of calving is nowadays considered normal (Pahl et al., 2014; Kovács et al., 2017). This phenomenon, together with the lack of energy and electrolytes due to decreased dry matter intake predisposes the cow to further postpartum diseases, such as milk fever, retained placenta or abomasal displacement (Goff and Horst, 1997).

Drenching is one of the methods that aim to restore or maintain ruminal function in the periparturient period. Several products are available that can be administered via drenching gun (Miyoshi et al., 2001), oesophageal tube (Schallenberger Gonçalves et al., 2015), or as a drinkable drench (McFadden et al., 2010). These products mainly contain glycogenic precursors, salts, and direct fed microbials (DFM) (probiotics, yeast).

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Glycogenic precursors (propylene glycol, glycerol, calcium propionate) serve as a source of energy. Propylene glycol is either metabolized in the rumen and is absorbed as propionate or propanol (Pehrson et al., 1998) which is converted into glucose by the liver, or it is absorbed through the rumen or gastrointestinal wall, and it is also metabolised into glucose in the liver (Kristensen and Raun, 2007; Rizos et al., 2008). Glycerol follows similar pathways. It can be metabolized by rumen microbes and enter the bloodstream as propionate to be used up in liver gluconeogenesis, or it passes through the wall of the rumen or small intestine and is converted into glucose or triacylglycerol in the liver (Kupczynski et al., 2020). Calcium propionate is hydrolysed into calcium and propionic acid in the rumen under acidic conditions, after which its components enter the bloodstream and the propionate is synthesised into glucose in the liver (Duplessis et al., 2017).

Simple salts are used in postpartum drenches because they supplement ions lost during calving and the onset of lactation. Calcium can be supplemented in several forms to decrease the risk of hypocalcaemia and milk fever. Goff and Horst (1993) have found that the oral administration of calcium chloride causes the quickest increase in plasma calcium, followed by calcium propionate and calcium carbonate, but it can also cause severe metabolic acidosis. Magnesium supplementation is fundamental in maintaining calcium homeostasis since it influences the absorption of dietary calcium (Enemark et al., 2009). Potassium is the main cation of the intracellular space and is essential in maintaining acid-base balance (Enemark et al., 2009).

The direct fed microbials play a role in the regulation of ruminal function. Probiotic feed additives gained popularity amid concerns of antimicrobial resistance to improve animal health and productivity (Allen et al., 2013). Probiotics can be classified as lactic acid producing (e.g., *Streptococcus bovis*, *Lactobacillus* spp., *Enterococcus* spp.) or lactic acid utilizing bacteria (*Selenomonas ruminantium*, *Megasphaera elsdenii*) and their number influences feed efficiency and rumen health via regulating the rumen pH (Nocek et al., 2002; Seo et al., 2010). Yeasts are known to cause the same effect on rumen pH as probiotics through their interactions with both lactate producing and lactate utilizing bacteria (Michalet-Doreau and Morand, 1996): they can moderate rumen acidosis by competing with *S. bovis* for resources, or by providing substrates and cofactors for lactate-utilizing bacteria (Retta, 2016).

Metabolic changes associated with NEB may be present as early as 6 weeks prepartum (Drackley et al., 2001). This might manifest as a lower-than-average rumination time before calving, which may influence the cows' adaptation to the metabolic requirements of early lactation. A study has found that animals with lower rumination times prepartum are more at risk of developing diseases postpartum than their counterparts with higher rumination times (Soriani et al., 2012). Thus, the aim of the present study was to explore the influence of postpartum drenching with a feed additive on the plasma concentration of important biochemical parameters while taking prepartum rumination

times into account. Rumination time and reticuloruminal pH data of the same experiment have been published previously (Lénárt et al., 2023).

## MATERIALS AND METHODS

The study was conducted in full compliance with the guidelines of the Animal Experimentation Committee (Budapest, Hungary).

### Animals

Two hundred Holstein-Friesian dairy cows (mean parity  $\pm$ SD:  $3.1 \pm 1.1$ ; range: 2–6) at Dózsa Agricultural Ltd.-Tass, Hungary were enrolled in the current study. They were all clinically healthy and in their dry period. The dairy operation has about 1000 Holstein-Friesian cows.

### Housing system

Dry cows were kept in a free stall barn in a large group (up to 80 animals). Three to five days before the expected calving date they were moved to one of five calving pens (approximately  $5 \times 7$  m of each). Each pen could house up to 6 animals. They also served as fresh cow pens for approximately the first five days after calving. Cows moving to the fresh cow pen on Day 5 were continuously replaced by prepartum animals, creating a dynamic system. Calving pens had a deep straw bedding. Prepartum cows were fed a prepartum total mixed ration (TMR) twice daily at approximately 06:00 and 17:00 h *ad libitum* containing a dietary forage-to-concentrate ratio of 78:22 on a dry matter (DM) basis. Postpartum cows were fed a fresh cow diet at approximately 08:00 and 17:00 h *ad libitum*. Water was also available *ad libitum*.

### Experimental design

Healthy cows entering the calving pen in the study period were fitted with Ruminact<sup>®</sup> HR-Tags (SCR Engineering Ltd., Netanya, Israel). Rumination time (RT) was recorded using a 2-min increments that were automatically summed and stored in 2-h intervals, as described and validated by Schirmann et al. (2009). Cows were randomly assigned into either the treatment (drenched) or to the control group based on their actual calving dates. Those in the drenched group received three treatments with the feed additive and technique detailed below (within 24 h after calving, and on the two subsequent days [Day 2 and Day 3]), after the morning milking and blood sampling. Control animals were managed the same way as the treatment group (blood was collected after the morning milking), but they were not drenched. Ruminact<sup>®</sup> HR-Tags were removed on Day 5 postpartum.

### Drenching

Reticuloruminal drenching treatment was done on Days 1, 2 and 3 using Sano Drenching Set, which contains an



orogastric tube, and a 40 L portable bucket and suction pump (Sano Modern Animal Nutrition Ltd., Csém, Hungary). The procedure was performed following the manufacturer's instructions. A feed additive mixture was used: 680 g calcium-propionate, 230 g magnesium-sulphate, 110 g potassium-chloride, 50 g sodium-chloride (Reanal Laboratory Chemicals Ltd., Budapest, Hungary), 230 g yeast (Europrotein Ltd., Verőce, Hungary) in approx. 25 L of lukewarm tap water. Each dose of the drenching mixture was prepared by hand and stored in individual bags at room temperature and used within a few days after preparation. The exact times of calving and every drenching were recorded.

### Blood sample collection and laboratory analyses

Blood samples were collected from all animals after the morning milking on Days 1, 2, 3, 7 and 12. The tail vein (*v. coccygea*) was punctured with a sterile 18G needle and blood was collected into two blood collection tubes (BD Vacutainer® K3E 3.6 mg 2.0 mL and BD Vacutainer® LH 68 I.U. 4.0 mL, [BD Hungary, Környe, Hungary]). The samples were placed in coolers and transported to the laboratory for analysis within two hours. The samples were centrifuged at 3,500 rpm for 5 min and the plasma was separated. The concentration/activity of biochemical parameters [aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), glucose, albumin, total protein, Na, K, total Ca, Cl, Mg, P, urea, creatinine] was measured using a biochemistry analyser (Olympus 640, Beckman Coulter Inc., Beckman Coulter Hungary Ltd., Budapest, Hungary). The concentration of  $\beta$ -hydroxybutyrate (BHB) was measured using  $\beta$ -hydroxybutyrate 21 FS kit (Diagnostic Systems GmbH, Holzheim, Germany), and the concentration of NEFA was measured using the NEFA FS kit (Diagnostic Systems GmbH, Holzheim, Germany). If an animal's blood sample was inadequate for analysis (coagulation, insufficient amount of plasma), the animal was excluded from the final analysis.

### Statistical analysis

The data were analysed using the R3.5.2 statistical software (R Core Team, 2018).

A 96-hour herd RT average was calculated using the sum of all study animals' RT in the last four days prepartum. This average was used as a threshold and the cows below it were categorized as "low rumination" and those above it as "high rumination" animals. These categories were merged with the drench/control assignments to create the final groups. Animals that had no 96 h of RT data available before calving were excluded from the herd average and the final analysis.

The data were checked for normality using the Shapiro-Wilk test. Due to uneven group populations, distribution of animals in the study groups was tested with two-sample proportion test.

To analyse the changes in each biochemical parameter, a generalized linear mixed model (GLMM) was used with the days after calving and study groups (by rumination and

drenching) as fixed effects, and cow ID as random effect. A GLM was created for each parameter to explore each day individually, where rumination, drenching and their interaction were used as factors.

One-way ANOVA and Tukey's *post hoc* test were performed to determine significant changes between days for the same parameter in each group. A probability value of  $P < 0.05$  was considered as statistically significant.

## RESULTS

One hundred and fifty-nine animals were found to fit the inclusion criteria for the final analysis. The study groups of "Low rumination control", "Low rumination drench", "High rumination control" and "High rumination drench" contained 34, 36, 38 and 51 animals, respectively. The number of cows in the four groups was considered statistically even ( $P > 0.05$ ).

### BHB, NEFA and glucose concentrations

The concentration of BHB was significantly higher in "Low rumination" animals compared to the "High rumination" ones (Table 1).

Table 2 shows the mean ( $\pm$ SD) plasma concentrations of BHB, NEFA, and glucose by day of lactation. A significant increase was found in the main values of BHB by days of lactation ( $P < 0.01$ ). NEFA showed the same changes ( $P < 0.01$ ). The glucose level did not show significant differences in the study groups, but it decreased significantly from Day 1 to Day 12 ( $P < 0.01$ ).

### Albumin and total protein concentrations

Blood total protein concentration was significantly higher in "Low rumination" animals ( $P < 0.01$ ) and significantly lower in the drenched groups ( $P < 0.01$ ) (Table 1). These changes were also observable individually on Days 3 and 7 for rumination, and on Day 2 for drenching (Table 2). Albumin decreased with days of lactation ( $P < 0.01$ ) while total protein showed a significant increase ( $P < 0.01$ ).

### Enzyme concentrations

In the GLMM, the activity of ALP and GGT was significantly higher ( $P < 0.01$ ) and ALT was significantly lower in "Low rumination" groups ( $P < 0.01$ ) (Table 1, Table 3). Both GGT and ALT were higher in drenched animals ( $P = 0.01$  and  $P = 0.02$ , respectively). These changes were observable individually on Days 1–3. The activity of AST and ALP was not different between the study groups during the period of drenching, but AST increased ( $P < 0.01$ ), and ALT, ALP and GGT decreased significantly with the days of lactation ( $P = 0.03$ ,  $P < 0.01$ ,  $P < 0.01$ , respectively).

### Urea and creatinine concentrations

The GLM showed that the plasma concentrations of both urea and creatinine were lower in drenched groups



**Table 1.** Cumulative effect of rumination, drenching and days of lactation (95% confidence intervals and *P*) on plasma concentrations/activity of measured biochemical parameters using generalised linear models. Rumination groups were based on the herd average in the 96 h before calving (high/low). Treatment groups were control and drench. Animals in the drench groups were treated three times after calving (Days 1, 2, and 3). Blood sampling was performed on Days 1, 2, 3, 7 and 12 after calving

	Rumination		Drenching		Days of lactation	
	Conf. int.	<i>P</i>	Conf. int.	<i>P</i>	Conf. int.	<i>P</i>
Beta-hydroxybutyrate (BHB)	0.022–0.234	0.02	–0.077–0.135	0.60	0.092–0.354	<0.01
Non-esterified fatty acids (NEFA)	–0.009–0.111	0.09	–0.060–0.060	0.99	0.038–0.258	<0.01
Glucose	–0.051–0.261	0.19	–0.0142–0.173	0.85	–1.301 to –0.929	<0.01
Albumin	–0.288–0.578	0.51	–0.459–0.406	0.90	–2.837 to –1.767	<0.01
Total protein	1.549–3.784	<0.01	–3.581 to –1.345	<0.01	1.362–4.107	<0.01
Aspartate transaminase (AST)	–9.035–4.440	0.50	–4.665–8.795	0.55	21.058–37.651	<0.01
Alanine aminotransferase (ALT)	–4.081 to –2.452	<0.01	0.166–1.794	0.02	–1.317–0.689	0.03
Alkaline phosphatase (ALP)	10.250–30.713	<0.01	–12.188–8.252	0.71	–78.031 to –52.840	<0.01
Gamma-glutamyl transferase (GGT)	1.852–4.222	<0.01	0.398–2.763	0.01	–0.044–2.879	<0.01
Urea	–0.274–0.086	0.31	–0.547 to –0.187	<0.01	–0.542 to –0.097	<0.01
Creatinine	0.831–4.845	0.01	–6.396 to –2.387	<0.01	–14.962 to –10.016	<0.01
Calcium	–0.078 to –0.018	<0.01	–0.049–0.011	0.22	0.093–0.167	<0.01
Magnesium	–0.096 to –0.045	<0.01	–0.034–0.017	0.51	–0.154 to –0.092	<0.01
Phosphate	–0.081–0.036	0.45	–0.101–0.015	0.15	–0.002–0.142	<0.01
Sodium	–1.104 to –0.048	0.03	–0.341–0.715	0.49	–3.534 to –2.230	<0.01
Potassium	–0.301 to –0.087	<0.01	–0.014 to –0.199	0.09	–0.091–0.172	0.53
Chloride	–0.612–0.390	0.66	0.712–1.713	<0.01	–3.825 to –2.589	<0.01

compared to the controls ( $P < 0.01$ ) (Table 1). This was also found Days 2 and 3 individually (Table 3). Urea and creatinine both decreased during the study period ( $P < 0.01$ ).

### Ion concentrations

The concentrations of calcium, magnesium, sodium and potassium were significantly lower in “Low rumination” animals (Table 1). This effect was also found individually on Day 12 for magnesium, and on Day 2 for potassium and chloride (Table 4). Chloride concentrations were significantly higher in drenched animals compared to controls ( $P < 0.01$ ). All ions except for potassium were significantly influenced by day of lactation, with the plasma concentration of total calcium, phosphate and magnesium increasing, and sodium and chloride concentrations decreasing during the study period ( $P < 0.01$ ).

## DISCUSSION

The periparturient period is associated with a change in several biochemical parameters. NEB is one of the major factors that play a role in these changes. NEB usually causes lipid mobilisation from the body's fat stores in the form of NEFA that is accumulated in the liver and contributes to the onset of ketosis (Ringseis et al., 2014). The liver also faces a higher demand for glucose to be used for lactose production in the udder, which results in metabolic stress (Ringseis et al., 2014). Cows can develop bacterial diseases (metritis, mastitis) in the periparturient period, that will result in increased levels of proinflammatory cytokines and the possible exposure to lipopolysaccharides that would induce an inflammation-like condition in the liver (Ringseis et al., 2014).

The response of the liver is the production of positive acute phase proteins, which in turn influences its capacity to produce other proteins, such as albumin or enzymes (Carroll et al., 2009). The aim of the present study was monitoring these changes in the biochemical parameters of the plasma of dairy cows in the periparturient period while evaluating the effect of a feed additive, delivered via drench on these parameters, controlling for previous rumination activity.

Excessive NEB is commonly diagnosed based on elevated levels of ketone bodies and NEFA (Ospina et al., 2010). The plasma concentration of BHB and NEFA increased significantly with days of lactation in the current study, the mean value of BHB slightly exceeding the cut-off value suggested by previous research (1.4 mmol L<sup>–1</sup> for metabolic diseases, Duffield et al., 2009) in the “Low rumination” drenched group on Day 12, although this average (1.46 mmol L<sup>–1</sup>) was coupled with high standard deviation (1.74 mmol L<sup>–1</sup>). While a cow is considered to be in a state of increased lipomobilisation above a NEFA concentration of 0.4 mmol L<sup>–1</sup> (Diaz Gonzalez et al., 2011), the cut-off point for NEFA for increased risk of ketosis was reported to be 0.82 mmol L<sup>–1</sup> (Cao et al., 2017). The mean value of the “Low rumination” control group was above this threshold on Day 2, and all study groups exceeded it on Days 7 and 12. Increased BHB and NEFA levels are nowadays considered a normal part of the metabolic changes around parturition (Duffield et al., 2009; Ringseis et al., 2014), and energy supplementation with glucogenic precursors is used to prevent an elevation that would put the cows at risk for diseases.

In the current study, plasma concentrations of BHB were not influenced by drenching. Previous works by other research groups have produced diverse results on the effect of calcium propionate on BHB. DeFrain et al. (2005) have found no influence of supplementing fresh cows with



Table 2. Plasma concentrations (mean  $\pm$  SD) of beta-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), glucose, albumin and total protein in the study groups. Low/high rumination was based on the herd average in the 96 h before calving. Animals in the drench groups were treated three times after calving (Days 1, 2, and 3). D1–D12 represent the days after calving. *P* values for the effects of rumination, drenching and their interaction obtained using linear models are also listed for each day. Superscripts signal statistically significant differences found using one-way ANOVA, between the days within study groups (to be viewed vertically) (*P* < 0.05)

		Low rumination control ( <i>n</i> = 34)	Low rumination drench ( <i>n</i> = 36)	High rumination control ( <i>n</i> = 38)	High rumination drench ( <i>n</i> = 51)	Effect of rumination ( <i>P</i> )	Effect of drenching ( <i>P</i> )	Rumination $\times$ drenching interaction ( <i>P</i> )
BHB (mmol L <sup>-1</sup> )	D1	0.87 $\pm$ 0.38	0.93 $\pm$ 0.57	0.75 $\pm$ 0.24	<sup>a</sup> 0.77 $\pm$ 0.24	0.18	0.89	0.67
	D2	0.90 $\pm$ 0.28	0.92 $\pm$ 0.44	0.86 $\pm$ 0.29	<sup>ab</sup> 0.79 $\pm$ 0.24	0.59	0.30	0.36
	D3	1.09 $\pm$ 0.69	1.16 $\pm$ 0.86	1.06 $\pm$ 0.49	<sup>ab</sup> 0.96 $\pm$ 0.72	0.85	0.54	0.46
	D7	1.20 $\pm$ 0.84	1.09 $\pm$ 0.71	1.08 $\pm$ 0.93	<sup>ab</sup> 1.02 $\pm$ 0.78	0.56	0.73	0.85
	D12	1.09 $\pm$ 0.77	1.46 $\pm$ 1.74	1.05 $\pm$ 0.99	<sup>b</sup> 1.17 $\pm$ 1.15	0.82	0.60	0.56
NEFA (mmol L <sup>-1</sup> )	D1	0.64 $\pm$ 0.43	0.73 $\pm$ 0.47	<sup>a</sup> 0.61 $\pm$ 0.37	<sup>a</sup> 0.51 $\pm$ 0.32	0.72	0.24	0.15
	D2	0.84 $\pm$ 0.54	0.78 $\pm$ 0.42	<sup>ab</sup> 0.69 $\pm$ 0.39	<sup>ab</sup> 0.73 $\pm$ 0.41	0.15	0.67	0.47
	D3	0.66 $\pm$ 0.38	0.69 $\pm$ 0.33	<sup>a</sup> 0.60 $\pm$ 0.31	<sup>ab</sup> 0.71 $\pm$ 0.39	0.54	0.18	0.53
	D7	0.92 $\pm$ 0.48	0.91 $\pm$ 0.49	<sup>b</sup> 0.94 $\pm$ 0.40	<sup>b</sup> 0.86 $\pm$ 0.46	0.85	0.42	0.60
	D12	0.83 $\pm$ 0.48	0.88 $\pm$ 0.44	<sup>b</sup> 0.90 $\pm$ 0.46	<sup>b</sup> 0.85 $\pm$ 0.42	0.56	0.64	0.53
Glucose (mmol L <sup>-1</sup> )	D1	<sup>b</sup> 4.04 $\pm$ 2.06	<sup>c</sup> 3.87 $\pm$ 0.89	<sup>b</sup> 4.06 $\pm$ 1.28	<sup>b</sup> 4.25 $\pm$ 1.99	0.97	0.62	0.53
	D2	<sup>a</sup> 3.07 $\pm$ 0.64	<sup>bc</sup> 3.47 $\pm$ 0.74	<sup>a</sup> 2.83 $\pm$ 0.67	<sup>a</sup> 3.03 $\pm$ 0.58	0.18	0.19	0.36
	D3	<sup>a</sup> 2.99 $\pm$ 0.78	<sup>ab</sup> 3.17 $\pm$ 0.77	<sup>a</sup> 2.97 $\pm$ 0.69	<sup>a</sup> 3.04 $\pm$ 0.63	0.90	0.68	0.67
	D7	<sup>a</sup> 2.99 $\pm$ 0.76	<sup>a</sup> 2.86 $\pm$ 0.58	<sup>a</sup> 2.93 $\pm$ 0.69	<sup>a</sup> 2.82 $\pm$ 0.62	0.76	0.51	0.97
	D12	<sup>ab</sup> 3.26 $\pm$ 0.64	<sup>a</sup> 2.79 $\pm$ 0.73	<sup>a</sup> 2.84 $\pm$ 0.70	<sup>a</sup> 2.56 $\pm$ 0.62	0.04	0.14	0.47
Albumin (g L <sup>-1</sup> )	D1	<sup>c</sup> 34.37 $\pm$ 3.40	<sup>b</sup> 34.39 $\pm$ 3.31	<sup>c</sup> 34.01 $\pm$ 2.34	<sup>d</sup> 34.06 $\pm$ 2.83	0.52	0.94	0.90
	D2	<sup>bc</sup> 33.40 $\pm$ 2.81	<sup>b</sup> 33.53 $\pm$ 3.09	<sup>c</sup> 33.51 $\pm$ 2.22	<sup>cd</sup> 32.80 $\pm$ 2.16	0.86	0.20	0.31
	D3	<sup>bc</sup> 32.92 $\pm$ 3.09	<sup>ab</sup> 32.80 $\pm$ 3.57	<sup>bc</sup> 32.59 $\pm$ 2.02	<sup>bc</sup> 32.51 $\pm$ 2.41	0.62	0.88	0.97
	D7	<sup>ab</sup> 31.25 $\pm$ 4.08	<sup>a</sup> 30.70 $\pm$ 3.30	<sup>a</sup> 30.60 $\pm$ 3.20	<sup>ab</sup> 31.05 $\pm$ 2.85	0.42	0.54	0.36
	D12	<sup>a</sup> 30.10 $\pm$ 4.46	<sup>a</sup> 30.93 $\pm$ 3.70	<sup>ab</sup> 31.04 $\pm$ 3.28	<sup>a</sup> 30.93 $\pm$ 3.24	0.28	0.88	0.42
Total protein (g L <sup>-1</sup> )	D1	<sup>a</sup> 75.52 $\pm$ 8.44	73.25 $\pm$ 7.58	<sup>a</sup> 73.44 $\pm$ 8.91	<sup>a</sup> 72.03 $\pm$ 7.11	0.27	0.41	0.74
	D2	<sup>ab</sup> 78.06 $\pm$ 6.61	75.77 $\pm$ 7.21	<sup>ab</sup> 75.30 $\pm$ 7.42	<sup>a</sup> 71.62 $\pm$ 6.61	0.09	0.01	0.54
	D3	<sup>ab</sup> 79.73 $\pm$ 8.13	76.18 $\pm$ 8.89	<sup>ab</sup> 74.30 $\pm$ 7.62	<sup>ab</sup> 74.01 $\pm$ 7.66	<0.01	0.75	0.25
		79.31 $\pm$ 7.08						
	D7	<sup>ab</sup> 79.31 $\pm$ 7.08	74.95 $\pm$ 7.85	<sup>ab</sup> 75.38 $\pm$ 7.68	<sup>a</sup> 72.39 $\pm$ 8.11	0.04	0.09	0.59
	D12	<sup>b</sup> 80.65 $\pm$ 7.05	78.53 $\pm$ 9.47	<sup>b</sup> 79.06 $\pm$ 8.93	<sup>b</sup> 77.16 $\pm$ 8.86	0.46	0.34	0.94



**Table 3.** Plasma activity (mean  $\pm$  SD) of aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and concentrations (mean  $\pm$  SD) of urea, and creatinine in the study groups. Low/high rumination was based on the herd average in the 96 h before calving. Animals in the drench groups were treated three times after calving (Days 1, 2, and 3). D1–D12 represent the days after calving. *P* values for the effects of rumination, drenching, and their interaction obtained using linear models are also listed for each day. Superscripts signal statistically significant differences found using one-way ANOVA, between the days within study groups (to be viewed vertically) (*P* < 0.05)

		Low rumination control ( <i>n</i> = 34)	Low rumination drench ( <i>n</i> = 36)	High rumination control ( <i>n</i> = 38)	High rumination drench ( <i>n</i> = 51)	Effect of rumination ( <i>P</i> )	Effect of drenching ( <i>P</i> )	Rumination $\times$ drenching interaction ( <i>P</i> )
AST (U/L)	D1	<sup>a</sup> 104.72 $\pm$ 30.01	<sup>a</sup> 101.18 $\pm$ 23.15	<sup>a</sup> 105.46 $\pm$ 33.94	<sup>a</sup> 110.21 $\pm$ 32.62	0.92	0.47	0.40
	D2	<sup>ab</sup> 115.23 $\pm$ 47.72	<sup>ab</sup> 123.80 $\pm$ 36.00	<sup>ab</sup> 112.03 $\pm$ 31.20	<sup>a</sup> 120.26 $\pm$ 25.00	0.70	0.27	0.98
	D3	<sup>ac</sup> 126.93 $\pm$ 61.39	<sup>ab</sup> 128.66 $\pm$ 39.06	<sup>ab</sup> 118.75 $\pm$ 31.38	<sup>ab</sup> 126.49 $\pm$ 41.12	0.44	0.42	0.67
	D7	<sup>c</sup> 151.18 $\pm$ 57.12	<sup>b</sup> 150.86 $\pm$ 68.00	<sup>bc</sup> 141.55 $\pm$ 53.14	<sup>c</sup> 156.43 $\pm$ 54.49	0.49	0.25	0.42
	D12	<sup>bc</sup> 146.07 $\pm$ 59.98	<sup>b</sup> 131.56 $\pm$ 44.75	<sup>c</sup> 160.05 $\pm$ 76.43	<sup>bc</sup> 149.38 $\pm$ 66.79	0.36	0.44	0.85
ALT (U/L)	D1	18.86 $\pm$ 5.12	19.01 $\pm$ 4.05	22.18 $\pm$ 6.34	23.56 $\pm$ 5.97	0.01	0.24	0.49
	D2	19.35 $\pm$ 6.19	20.17 $\pm$ 3.89	21.80 $\pm$ 5.29	24.03 $\pm$ 5.33	0.05	0.05	0.40
	D3	19.86 $\pm$ 7.76	20.12 $\pm$ 4.46	20.85 $\pm$ 4.25	24.20 $\pm$ 5.70	0.46	0.01	0.09
	D7	19.61 $\pm$ 6.00	18.58 $\pm$ 5.54	20.25 $\pm$ 6.50	22.55 $\pm$ 6.53	0.67	0.09	0.10
	D12	18.00 $\pm$ 5.51	17.29 $\pm$ 4.63	21.62 $\pm$ 7.27	21.63 $\pm$ 6.72	0.02	0.99	0.72
ALP(U/L)	D1	<sup>c</sup> 227.48 $\pm$ 82.51	<sup>b</sup> 245.21 $\pm$ 94.64	<sup>d</sup> 223.21 $\pm$ 72.49	<sup>c</sup> 225.53 $\pm$ 89.38	0.83	0.90	0.58
	D2	<sup>c</sup> 217.42 $\pm$ 114.28	<sup>ab</sup> 214.04 $\pm$ 68.96	<sup>cd</sup> 201.74 $\pm$ 57.42	<sup>b</sup> 174.79 $\pm$ 69.03	0.40	0.11	0.35
	D3	<sup>bc</sup> 197.29 $\pm$ 82.92	<sup>a</sup> 188.04 $\pm$ 65.69	<sup>bc</sup> 178.91 $\pm$ 58.57	<sup>b</sup> 166.39 $\pm$ 60.92	0.25	0.39	0.88
	D7	<sup>ab</sup> 147.17 $\pm$ 69.35	<sup>a</sup> 165.01 $\pm$ 67.76	<sup>ab</sup> 146.95 $\pm$ 60.49	<sup>a</sup> 128.99 $\pm$ 44.97	0.99	0.18	0.07
	D12	<sup>a</sup> 122.36 $\pm$ 56.21	<sup>a</sup> 164.78 $\pm$ 101.50	<sup>a</sup> 133.13 $\pm$ 62.84	<sup>a</sup> 116.29 $\pm$ 40.15	0.50	0.25	0.01
GGT (U/L)	D1	15.94 $\pm$ 10.66	20.19 $\pm$ 8.23	<sup>a</sup> 11.25 $\pm$ 7.06	14.73 $\pm$ 7.14	0.02	0.05	0.77
	D2	15.76 $\pm$ 8.44	16.80 $\pm$ 7.65	<sup>a</sup> 13.88 $\pm$ 5.69	14.33 $\pm$ 6.88	0.27	0.78	0.80
	D3	16.31 $\pm$ 11.22	18.83 $\pm$ 6.53	<sup>a</sup> 13.10 $\pm$ 5.90	16.32 $\pm$ 7.13	0.09	0.06	0.78
	D7	18.86 $\pm$ 12.32	18.98 $\pm$ 8.40	<sup>ab</sup> 15.18 $\pm$ 7.58	14.90 $\pm$ 8.35	0.11	0.89	0.90
	D12	18.48 $\pm$ 8.39	21.49 $\pm$ 7.97	<sup>b</sup> 19.95 $\pm$ 10.73	18.20 $\pm$ 8.04	0.49	0.37	0.10
Urea (mmol L <sup>-1</sup> )	D1	<sup>b</sup> 5.85 $\pm$ 1.38	<sup>bc</sup> 5.27 $\pm$ 1.17	<sup>ab</sup> 5.54 $\pm$ 1.30	5.23 $\pm$ 1.12	0.29	0.24	0.50
	D2	<sup>b</sup> 5.78 $\pm$ 1.54	<sup>c</sup> 5.48 $\pm$ 1.50	<sup>b</sup> 5.84 $\pm$ 1.17	5.28 $\pm$ 1.14	0.84	0.05	0.54
	D3	<sup>b</sup> 5.68 $\pm$ 1.66	<sup>ac</sup> 5.08 $\pm$ 1.40	<sup>b</sup> 5.91 $\pm$ 1.22	5.07 $\pm$ 1.20	0.47	0.01	0.57
	D7	<sup>ab</sup> 5.02 $\pm$ 1.51	<sup>a</sup> 4.32 $\pm$ 1.16	<sup>a</sup> 4.87 $\pm$ 1.06	4.84 $\pm$ 1.14	0.61	0.94	0.09
	D12	<sup>a</sup> 4.54 $\pm$ 1.30	<sup>ab</sup> 4.56 $\pm$ 0.96	<sup>a</sup> 4.84 $\pm$ 1.43	5.03 $\pm$ 1.11	0.30	0.49	0.67
Creatinine (μmol L <sup>-1</sup> )	D1	<sup>b</sup> 105.21 $\pm$ 13.07	<sup>b</sup> 106.28 $\pm$ 17.54	<sup>b</sup> 100.53 $\pm$ 14.39	<sup>c</sup> 101.94 $\pm$ 15.18	0.20	0.66	0.94
	D2	<sup>b</sup> 105.90 $\pm$ 12.47	<sup>a</sup> 94.55 $\pm$ 16.07	<sup>b</sup> 101.65 $\pm$ 11.72	<sup>ab</sup> 89.18 $\pm$ 14.77	0.20	<0.01	0.80
	D3	<sup>b</sup> 101.24 $\pm$ 11.58	<sup>a</sup> 93.49 $\pm$ 16.48	<sup>b</sup> 102.42 $\pm$ 12.13	<sup>b</sup> 90.38 $\pm$ 14.13	0.72	<0.01	0.34
	D7	<sup>a</sup> 88.60 $\pm$ 14.70	<sup>a</sup> 85.29 $\pm$ 14.88	<sup>a</sup> 86.47 $\pm$ 10.57	<sup>ab</sup> 86.22 $\pm$ 12.99	0.51	0.93	0.48
	D12	<sup>a</sup> 84.51 $\pm$ 15.06	<sup>a</sup> 85.96 $\pm$ 12.69	<sup>a</sup> 82.09 $\pm$ 15.30	<sup>a</sup> 81.91 $\pm$ 11.37	0.46	0.95	0.71



**Table 4.** Plasma concentrations (mean  $\pm$  SD) of total calcium, magnesium, phosphate, sodium, potassium, and chloride in the study groups. Low/high rumination was based on the herd average in the 96 h before calving. Animals in the drench groups were treated three times after calving (Days 1, 2 and 3). D1–D12 represent the days after calving. *P* values for the effects of rumination, drenching and their interaction obtained using linear models are also listed for each day. Superscripts signal statistically significant differences found using one-way ANOVA, between the days within study groups (to be viewed vertically) ( $P < 0.05$ )

		Low rumination control ( $n = 34$ )	Low rumination drench ( $n = 36$ )	High rumination control ( $n = 38$ )	High rumination drench ( $n = 51$ )	Effect of rumination ( <i>P</i> )	Effect of drenching ( <i>P</i> )	Rumination $\times$ drenching interaction ( <i>P</i> )
Calcium (mmol L <sup>-1</sup> )	D1	<sup>ab</sup> 2.08 $\pm$ 0.25	<sup>a</sup> 2.01 $\pm$ 0.28	<sup>a</sup> 2.10 $\pm$ 0.27	<sup>a</sup> 2.11 $\pm$ 0.22	0.72	0.83	0.33
	D2	<sup>a</sup> 2.04 $\pm$ 0.28	<sup>a</sup> 2.08 $\pm$ 0.22	<sup>a</sup> 2.07 $\pm$ 0.24	<sup>a</sup> 2.09 $\pm$ 0.020	0.54	0.78	0.74
	D3	<sup>bc</sup> 2.21 $\pm$ 0.32	<sup>ab</sup> 2.08 $\pm$ 0.19	<sup>ab</sup> 2.18 $\pm$ 0.24	<sup>a</sup> 2.16 $\pm$ 0.15	0.49	0.69	0.13
	D7	<sup>c</sup> 2.24 $\pm$ 0.13	<sup>bc</sup> 2.21 $\pm$ 0.13	<sup>bc</sup> 2.31 $\pm$ 0.21	<sup>b</sup> 2.30 $\pm$ 0.17	0.08	0.70	0.86
	D12	<sup>c</sup> 2.29 $\pm$ 0.15	<sup>c</sup> 2.29 $\pm$ 0.17	<sup>c</sup> 2.36 $\pm$ 0.20	<sup>b</sup> 2.34 $\pm$ 0.16	0.12	0.67	0.80
Magnesium (mmol L <sup>-1</sup> )	D1	<sup>c</sup> 1.01 $\pm$ 0.19	<sup>b</sup> 1.00 $\pm$ 0.18	<sup>b</sup> 1.06 $\pm$ 0.20	<sup>b</sup> 1.09 $\pm$ 0.22	0.33	0.48	0.53
	D2	<sup>bc</sup> 0.98 $\pm$ 0.20	<sup>ab</sup> 0.89 $\pm$ 0.20	<sup>b</sup> 1.04 $\pm$ 0.16	<sup>ab</sup> 1.00 $\pm$ 0.22	0.15	0.25	0.58
	D3	<sup>ac</sup> 0.93 $\pm$ 0.20	<sup>a</sup> 0.86 $\pm$ 0.21	<sup>ab</sup> 0.96 $\pm$ 0.17	<sup>a</sup> 0.97 $\pm$ 0.23	0.51	0.80	0.25
	D7	<sup>a</sup> 0.83 $\pm$ 0.12	<sup>a</sup> 0.84 $\pm$ 0.14	<sup>a</sup> 0.86 $\pm$ 0.016	<sup>a</sup> 0.89 $\pm$ 0.10	0.45	0.23	0.46
	D12	<sup>ab</sup> 0.88 $\pm$ 0.13	<sup>ab</sup> 0.90 $\pm$ 0.15	<sup>ab</sup> 0.96 $\pm$ 0.15	<sup>a</sup> 0.97 $\pm$ 0.14	0.01	0.77	0.79
Phosphate (mmol L <sup>-1</sup> )	D1	<sup>ab</sup> 1.80 $\pm$ 0.38	<sup>ab</sup> 1.77 $\pm$ 0.47	1.73 $\pm$ 0.42	<sup>ab</sup> 1.80 $\pm$ 0.43	0.45	0.42	0.43
	D2	<sup>ab</sup> 1.84 $\pm$ 0.50	<sup>a</sup> 1.61 $\pm$ 0.42	1.76 $\pm$ 0.44	<sup>a</sup> 1.59 $\pm$ 0.39	0.45	0.06	0.70
	D3	<sup>b</sup> 2.07 $\pm$ 0.42	<sup>ab</sup> 1.70 $\pm$ 0.037	1.94 $\pm$ 0.45	<sup>a</sup> 1.65 $\pm$ 0.45	0.20	<0.01	0.55
	D7	<sup>ab</sup> 1.83 $\pm$ 0.37	<sup>b</sup> 1.93 $\pm$ 0.30	1.91 $\pm$ 0.25	<sup>c</sup> 2.21 $\pm$ 0.41	0.35	<0.01	0.08
	D12	<sup>a</sup> 1.78 $\pm$ 0.36	<sup>b</sup> 1.88 $\pm$ 0.30	1.88 $\pm$ 0.33	<sup>b</sup> 1.94 $\pm$ 0.36	0.21	0.47	0.67
Sodium (mmol L <sup>-1</sup> )	D1	<sup>bc</sup> 140.93 $\pm$ 3.60	<sup>c</sup> 143.42 $\pm$ 4.18	<sup>b</sup> 142.68 $\pm$ 2.84	<sup>d</sup> 144.13 $\pm$ 3.79	0.05	0.07	0.38
	D2	<sup>bc</sup> 140.84 $\pm$ 3.39	<sup>bc</sup> 141.38 $\pm$ 3.68	<sup>b</sup> 142.49 $\pm$ 3.12	<sup>c</sup> 141.66 $\pm$ 3.51	0.04	0.26	0.22
	D3	<sup>c</sup> 141.91 $\pm$ 4.34	<sup>bc</sup> 141.31 $\pm$ 3.90	<sup>b</sup> 142.32 $\pm$ 3.00	<sup>bc</sup> 140.61 $\pm$ 3.17	0.63	0.03	0.34
	D7	<sup>ab</sup> 138.61 $\pm$ 4.40	<sup>ab</sup> 139.46 $\pm$ 4.27	<sup>a</sup> 139.62 $\pm$ 3.56	<sup>ab</sup> 139.20 $\pm$ 3.53	0.29	0.63	0.32
	D12	<sup>a</sup> 137.65 $\pm$ 4.22	<sup>a</sup> 137.56 $\pm$ 3.77	<sup>a</sup> 137.91 $\pm$ 3.48	<sup>a</sup> 138.55 $\pm$ 4.22	0.79	0.46	0.57
Potassium (mmol L <sup>-1</sup> )	D1	5.07 $\pm$ 0.80	4.82 $\pm$ 0.56	5.18 $\pm$ 0.80	<sup>a</sup> 4.93 $\pm$ 0.84	0.57	0.14	0.99
	D2	4.75 $\pm$ 0.59	5.00 $\pm$ 0.76	5.19 $\pm$ 0.88	<sup>ab</sup> 5.18 $\pm$ 0.65	0.01	0.97	0.28
	D3	4.83 $\pm$ 0.74	4.99 $\pm$ 0.75	5.10 $\pm$ 0.79	<sup>ab</sup> 5.11 $\pm$ 0.71	0.13	0.95	0.53
	D7	4.79 $\pm$ 0.68	4.95 $\pm$ 0.82	4.90 $\pm$ 0.73	<sup>ab</sup> 5.13 $\pm$ 0.68	0.55	0.15	0.74
	D12	4.86 $\pm$ 0.78	5.08 $\pm$ 0.80	4.93 $\pm$ 0.071	<sup>b</sup> 5.40 $\pm$ 0.67	0.70	0.01	0.33
Chloride (mmol L <sup>-1</sup> )	D1	<sup>b</sup> 102.80 $\pm$ 2.96	<sup>b</sup> 103.70 $\pm$ 3.38	<sup>c</sup> 103.01 $\pm$ 3.26	<sup>b</sup> 103.33 $\pm$ 2.86	0.78	0.62	0.57
	D2	<sup>b</sup> 101.16 $\pm$ 2.90	<sup>b</sup> 103.94 $\pm$ 2.71	<sup>bc</sup> 102.69 $\pm$ 2.92	<sup>b</sup> 103.78 $\pm$ 2.98	0.03	0.08	0.07
	D3	<sup>b</sup> 100.36 $\pm$ 3.03	<sup>b</sup> 103.73 $\pm$ 2.66	<sup>b</sup> 100.73 $\pm$ 2.79	<sup>b</sup> 103.37 $\pm$ 3.24	0.60	<0.01	0.45
	D7	<sup>a</sup> 97.32 $\pm$ 4.78	<sup>a</sup> 98.58 $\pm$ 4.02	<sup>a</sup> 98.96 $\pm$ 3.88	<sup>a</sup> 97.60 $\pm$ 3.24	0.45	0.61	0.19
	D12	<sup>a</sup> 96.73 $\pm$ 4.31	<sup>a</sup> 96.96 $\pm$ 4.66	<sup>a</sup> 96.96 $\pm$ 4.34	<sup>a</sup> 97.33 $\pm$ 4.47	0.83	0.70	0.93



calcium propionate on the concentration of BHB, although in that study the calcium propionate was mixed into the upper third of the TMR. Administered two times in a paste containing 37 g of calcium and 134 g of propionate, the treatment had no benefit on BHB concentrations in Holstein-Friesian cows (Goff et al., 1996). Stokes and Goff (2001) have published similar results after the oral administration of calcium propionate. Delivered in a drench, 700 g of calcium propionate did improve energy balance in another experiment, producing significantly lower BHB levels in treated animals compared to controls (Enemark et al., 2009). Liu et al. (2010) have also found a decrease in BHB with an increasing dose of calcium propionate supplemented to dairy cows during early lactation. In a review, it has been hypothesised that the lack of response to calcium propionate supplementation might be due to its relatively small addition to the volume that the rumen produces (Overton and Waldron, 2004). Also, differences in breed, metabolic state or feed composition might cause different reactions (Liu et al., 2010).

The plasma concentration of NEFA was also not significantly different among the study groups, although all averages signalled an increased lipomobilisation ( $>0.4 \text{ mmol L}^{-1}$ ) (Diaz Gonzalez et al., 2011). Previously, most researchers have published similar results. Goff et al. (1996) have found no significant decrease in the plasma concentration of NEFA after oral administration of calcium propionate paste. Other authors have also reported that supplementation with calcium propionate, mixed into the TMR (McNamara and Valdez, 2005), delivered via oesophageal tube (Kara et al., 2009; Zhang et al., 2022), or combined with a propylene glycol drench (Peralta et al., 2011) did not influence the concentration of NEFA in the plasma of postpartum dairy cows. There are, however, results that support the positive effect of calcium propionate on the energy balance. Mandevu et al. (2003) have found a tendency for lower NEFA concentration after supplementing the feed of dairy cows with calcium propionate. A mixture of calcium propionate and long-chain fatty acids have also been found to decrease NEFA levels significantly (DeFrain et al., 2005), while Liu et al. (2010) have reported that a more pronounced decrease can be achieved by increasing the amount of calcium propionate administered. This discrepancy indicates that, just like BHB, NEFA might be influenced by multiple factors.

The plasma concentration of glucose decreased continuously during the study period and became significantly lower than the value at calving on Day 2 in all groups except for the “Low rumination” drench, where the decrease did not reach statistical significance until Day 3. This can be explained by the increased demand associated with the onset of lactation (Ringseis et al., 2014), or with a compromised liver function resulting from NEB (Diaz Gonzalez et al., 2011). Plasma levels of the glucose were not affected by the treatment. This is in agreement with the results of previous studies (McNamara and Valdez, 2005; Kara et al., 2009). In another experiment (Liu et al., 2010), a significant difference has been found between the plasma glucose concentrations

of cows treated with calcium propionate and the controls. In that study, the averages of multiple samples have been used to correct for possible sampling errors.

In the current study, the plasma concentration of albumin decreased significantly with days of lactation, while the total protein showed an increase. The former finding is in accordance with previous results (Tóthová et al., 2008), where the albumin level was the lowest four weeks after calving. Piccione et al. (2011) have also detected a slight drop in the levels of albumin between calving and seven days postpartum. Albumin is the main osmotic component of the blood and its levels decline during episodes of stress with the increase of acute phase proteins (Tóthová et al., 2008; Ringseis et al., 2014), which might explain its changes in the periparturient period. Researchers have reported a postpartum decrease of total protein concentration (Piccione et al., 2011), which conflicts with the results of the present experiment. Others have found that lower total protein after calving is correlated with a low dietary cation-anion diet fed during dry-off (Grünberg et al., 2011), which was not applied in the current trial. In contrast, Tóthová et al. (2008) detected a steady elevation of total protein after calving. The increase in the plasma concentration of total protein can be explained by the increase of globulin fractions after calving (Rowlands et al., 1975). In our present study, the total protein concentration was significantly lower in the drenched groups than in the controls. This might be due to the large volume of water administered as part of the drenching solution that served to rehydrate the animals (Guterbrock, 2004).

AST showed a significant increase, while ALT, ALP and GGT a significant decrease during the study period. The changes in AST are in accordance with the results of Stojevic et al. (2005), who have found that the activity of AST is the lowest during dry-off, and it elevates in early lactation. The increase in AST activity can be a result of impaired liver function due to damage of hepatocytes during NEB (Zhang et al., 2022). In contrast with the current results, a previous study has found no changes in the plasma activities of ALT and GGT during the transition period (Stojevic et al., 2005). ALP consists of multiple isoenzymes, specific for the liver, intestine, bone or placenta during pregnancy (Mohebbi et al., 2010). The decrease in ALP levels after calving might be due to lower levels of bone specific ALP consistent with the resorption of bone tissue (Kim et al., 2010), or to the detachment of the placenta that would supply another isoenzyme. This decrease has been detected by others (Jonsson et al., 2013), but in that experiment, no significant difference was found between the samples taken at calving and around Day 15. Both ALT and GGT activities were higher in drenched groups compared to controls. Zhang et al. (2022) have found a significant decrease in ALT activity when supplementing cows with 350 g of calcium propionate in an oral drench from calving to Day 35 postpartum. They have also detected an increase in AST activity and a decrease in ALP activity as a result of drenching with 500 g of calcium propionate, therefore they have hypothesised that 500 g or more of calcium propionate might be



detrimental to liver function, which might be the cause of elevated ALT and GGT activities in drenched groups (Zhang et al., 2022).

Urea and creatinine showed a significant decrease from Day 1 to Day 12. Urea is produced in the liver from ammonia and amino acids (Lapierre and Lobley, 2001). Blood urea may be used in metabolic processes, be excreted by the kidneys, or be secreted into the saliva and re-enter the gastrointestinal tract during feeding and rumination (Lapierre and Lobley, 2001). Decreased plasma urea levels after calving can be explained both by increased feed intake and rumination, and by higher demand due to metabolic changes associated with milk production. Creatinine is formed non-enzymatically from creatine in the skeletal muscle of mammals and is indicative of changes in muscle mass (Wyss and Kaddurah-Daouk, 2000). Its concentration is increased in dehydration and with the decrease of glomerular filtration rate (Wyss and Kaddurah-Daouk, 2000). In periparturient dairy cows, mobilisation of skeletal muscle due to NEB has been documented, and the decreased muscle mass results in lower plasma concentrations of creatinine (Kokkonen et al., 2005; Megahed et al., 2019). Both urea and creatinine showed lower levels in drenched animals compared to controls. Kass et al. (2013) have published similar results regarding plasma urea concentrations after supplementing dairy cows with glycerol for 21 days postpartum. This might be an indicator of a more efficient protein metabolism in animals treated with glycogenic precursors.

The total calcium concentration in the plasma increased significantly during our study. In the periparturient period, most dairy cows are affected by some level of hypocalcaemia. This is the result of calcium being secreted into the udder to support the beginning of the milk production, while the intestinal absorption and mobilization from the bone are delayed (Goff and Horst, 1997). The threshold for subclinical hypocalcaemia is  $< 2 \text{ mmol L}^{-1}$  (Goff, 2008). In the current study, none of the study groups' averages decreased below this level and all had a mean total calcium concentration of  $> 2.2 \text{ mmol L}^{-1}$  by Day 7, although drenching had no significant effect on total calcium concentrations. Goff et al. (1996), Stokes and Goff (2001) and Zhang et al. (2022) have reported the same results, although several studies have found a decrease in the incidence of milk fever after the administration of calcium propionate (Goff et al., 1996; Pehrson et al., 1998; Kara et al., 2009). The lack of a significant increase in total calcium concentrations after drenching can be explained by the relatively less efficient absorption of calcium ions from the rumen compared to the small intestine (Kara, 2013). This is because the rumen juice rapidly dilutes the concentration of calcium below the level needed for passive transport (Kara, 2013). The plasma concentration of magnesium and phosphorus also increased from Day 1 to Day 12. This might be the result of increased resorption from bone reserves to provide for the needs of milk production. The decrease of the concentration of sodium and chloride can also be explained by increased demand of the udder. Only the plasma concentration of

chloride was affected by drenching. This could be due to the feed additive containing two chloride salts (potassium and sodium), and their effect to increase the chloride concentration and absorption from the rumen fluid.

One of the key aspects of our current study was to explore the effect of lower-than-average RT in the days before calving on the plasma concentrations of biochemical parameters. The postpartum levels of almost half of the measured metabolites, enzymes and ions were affected by prepartum ruminal function. BHB, total protein, ALP, GGT and creatinine were higher in the "Low rumination" groups after calving. The higher plasma concentration of BHB is an indicator of a decreased oxidation capacity of the liver (Churakov et al., 2021). Though increased lipomobilisation was present in all study groups, the effect of NEB was more severe in "Low rumination" animals. Soriani et al. (2012, 2013) have detected a similar relationship between RT and BHB. Liver activity/functionality index is also reduced in animals with lower RT postpartum (Soriani et al., 2012; Calamari et al., 2014), which might explain the higher plasma activities of ALP and GGT, although Soriani et al. (2012) have reported a lower GGT activity in groups with lower RT. Higher total protein concentration after calving in Low RT groups conflict with previous research (Calamari et al., 2014), but it might be the result of the mild dehydration that occurs after calving along with higher concentrations of creatinine (Enemark et al., 2009).

The current study also revealed lower ALT activity and lower calcium, magnesium and potassium concentrations in the plasma of "Low rumination" animals after calving. The decreased ALT activity compared to that in the "High rumination" groups cannot be explained easily, but it might be due to the higher performance of the "High rumination" animals (Calamari et al., 2014), which would suggest an increased activity of liver metabolism. Soriani et al. (2013) have also reported lower plasma magnesium concentrations in cows with lower RT, which they attributed to the result of decreased dry matter intake and subsequent higher fluid content in the rumen that would cause relatively lower ion concentrations in the rumen fluid, and thus impede their absorption. This might also be the reason for lower plasma sodium and potassium concentrations in the "Low rumination" groups. Lower plasma calcium levels in the periparturient period have been found to be associated with lower rumination rates (Goff et al., 2020), but in the previous study no prepartum RTs were recorded beyond 24 h before calving. These results indicate that prepartum RT significantly influences the postpartum biochemical profiles of dairy cows.

In conclusion, post partum drenching with the current feed additive affected the plasma concentrations and activities of total protein, ALT, GGT, urea, creatinine, and chloride. Lower than average prepartum RT influenced the levels of BHB, total protein, most liver enzymes, creatinine and ions including calcium and magnesium. The day of lactation had an effect on all parameters except for potassium. The current study found no major improvement on



dairy cows' plasma biochemistry caused by drenching in the days after calving.

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