



Effect of Feeding Anionic Ration during the Late Prepartum Period on Performance and Metabolic Profile in the Early Postpartum Period in Holstein and Simmental Dairy Cows

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Article History: 26-098 Received: 27-3-26 Revised: 30-5-26 Accepted: 1-6-26 Online First: 23-Jun-26

ABSTRACT

Hypocalcemia, a prevalent metabolic disorder in dairy cattle, particularly around parturition, significantly impacts early lactation. This study investigated the effects of prepartal anionic salt cocktail supplementation on early postpartum performance and metabolic profile in multiparous Holstein and Simmental cows. Thirty-two multiparous (≥ 3 lactations) Holstein and Simmental cows were initially enrolled at 23 days prior to projected parturition, and 22 cows completed the experiment and were included in the final analyses. They received daily supplementation of either 1 kg wheat bran (control) or 1 kg pelleted anionic salt product until calving. Post-calving, all cows transitioned to a standard early lactation diet until 28 days postpartum. DMI, milk yield, urine pH, and serum metabolic indices were measured. Urine and blood samples were collected prepartum, with blood sampling continuing until 28 days postpartum for metabolic profiling. Anionic salt feeding significantly decreased prepartum DMI (11.25 vs. 11.78 kg/d, $P < 0.0001$) but tended to increase postpartum DMI (25.78 vs. 25.64 kg/d; $P = 0.0530$). Urine pH declined significantly in treated cows (7.27 vs. 8.01; $P < 0.0001$), with a more pronounced effect observed in Simmental cows. While prepartum anionic feeding did not significantly alter serum PTH levels, breed-specific differences were evident in serum calcium and magnesium concentrations. Simmental cows showed greater postpartum DMI, a more pronounced reduction in urine pH, and differing serum calcium responses between breeds following anionic feeding, whereas overall postpartum serum calcium concentrations were higher in Holstein cows. These findings suggest possible breed-related differences in response to prepartal anionic salt supplementation under field conditions; however, further studies with larger and more balanced cohorts and varying DCAD levels are warranted to confirm these observations.

Keywords: Anionic salt, Dairy cows, Hypocalcemia, Metabolic profile.

INTRODUCTION

Clinical hypocalcemia is a metabolic disorder that can occur within 72 hrs after parturition, especially in high-yielding and multiparous cows. While clinical hypocalcemia affects approximately 5–10% of cows, subclinical hypocalcemia is far more prevalent, impacting 40–50% of cows in some herds. Both forms adversely affect health and performance by predisposing cows to primary (dystocia, retained placenta, abomasum displacement) and secondary (metritis, mastitis, ketosis, etc.) postpartum problems, as well as poor lactation and fertility performances (Goff 2008; Reinhardt et al. 2011).

Blood calcium (Ca) levels in cows range from 8.5–10 mg/dL during prepartum and decrease to 8 mg/dL at

parturition. Subclinical hypocalcemia occurs when the blood Ca level falls around 5.5–8.0 mg/dL, and clinical hypocalcemia occurs when it drops below 5.5 mg/dL (Reinhardt et al. 2011). The physiological decrease in blood calcium concentration at calving results from the sudden onset of lactation and the associated calcium demand for colostrum and milk synthesis. Parathyroid hormone (PTH) plays a central role in restoring calcium homeostasis by stimulating bone resorption and activating vitamin D to enhance intestinal calcium absorption. However, the effectiveness of PTH can be markedly reduced in cows experiencing metabolic alkalosis, which impairs receptor sensitivity (Goff et al. 2004). Metabolic alkalosis is commonly induced by prepartum diets with a high dietary cation-anion difference (DCAD),

Cite This Article as: Simsek U and Hayirli A, 2026. Effect of feeding anionic ration during the late prepartum period on performance and metabolic profile in the early postpartum period in holstein and simmental dairy cows. International Journal of Veterinary Science 15(4): 1067–1075. <https://doi.org/10.47278/journal.ijvs/2026.095>

often resulting from high potassium content in forages such as alfalfa and grass silage (Goff and Horst 2003b; Goff et al. 2007).

Prevention of hypocalcemia is more important and effective than treatment to avoid primary and secondary postpartum health problems as well as fertility and lactation losses (Oetzel 2015; Norouzi et al. 2020). Feeding anionic salts during the close-up period has emerged as an effective nutritional strategy to reduce the DCAD of the diet, thereby inducing a mild compensated metabolic acidosis. This metabolic shift enhances tissue responsiveness to PTH, promoting calcium mobilization and reducing the incidence of both clinical and subclinical hypocalcemia (Goff et al. 2004; Norouzi et al. 2020). Numerous studies confirm that reducing dietary DCAD to levels between -50 to -150 mEq/kg dry matter (DM) is beneficial in improving calcium status during the transition period (Oetzel 2012; Weich et al. 2013). However, the success of this approach is often limited by the unpalatable nature of anionic salts, which may decrease DM intake (DMI) and offset the potential benefits (Joyce et al. 1997; Vagnoni and Oetzel 1998; Wu et al. 2008). Approaches such as pelleting and molasses inclusion have been proposed to improve palatability (Seifi et al. 2010). While molasses contains relatively high potassium levels that could increase DCAD, its contribution is likely negligible given its low inclusion rate and positive impact on palatability; the overall acidifying effect of anionic salts far outweighs any DCAD-increasing effect from molasses.

Most existing studies evaluating the efficacy of anionic salt supplementation have been conducted in Holstein cows. However, differences in physiological and metabolic characteristics between breeds may influence their responsiveness to dietary acidification. Simmental cows, as a dual-purpose breed, may exhibit distinct mineral metabolism and acid-base regulation compared to Holsteins. In addition to physiological evaluation, improving the palatability and intake of anionic salts is critical for their practical application. Therefore, in this study, an anionic salt cocktail was formulated in pelleted form using wheat bran, flaked corn, and molasses to improve intake and reduce refusal. The study aimed to compare the early postpartum metabolic responses and DMI between Holstein and Simmental cows in response to prepartum supplementation with this pelleted anionic salt. It was hypothesized that breed-specific differences in calcium homeostasis and DMI would result in differential responses to dietary acidification, and that the pelleted form would support acceptable intake during the close-up period.

MATERIALS AND METHODS

Experimental design and management

The study was conducted at the Dutpinar commercial dairy farm (Cayol, Akcadag, Malatya). All animal procedures were approved by the Ataturk University Ethics Committee (2021, #75296309-050.01.04-E.2100037077). The experiment initially included 32 clinically healthy, pregnant, multiparous Holstein and Simmental cows (≥ 3 parity; 650 ± 65 kg BW). On day -23 relative to the expected calving date, cows were blocked by breed and previous 305-day mature equivalent milk yield and randomly assigned to one of two treatment groups, each consisting of 8 Holstein

and 8 Simmental cows. Cows were fed a total mixed ration (TMR) top-dressed with either 1 kg wheat bran (control) or 1 kg pelleted anionic salt supplement (1 kg product containing 55 g NH_4Cl , 55 g MgSO_4 , 55 g CaCl_2 , wheat bran, flaked corn, and molasses) per cow daily until calving. After parturition, all cows were switched to an early lactation TMR until day 28 postpartum. The isocaloric and isonitrogenous rations (Table 1) were formulated according to NRC (2001) recommendations. Cows were housed and fed in groups according to treatment. DMI was calculated on a group basis by dividing the total daily feed consumption by the number of cows within each group. A total of 22 cows completed the experiment (Control: 5 Simmental and 6 Holstein; Anionic salt: 7 Simmental and 4 Holstein). Ten cows were excluded due to earlier-than-expected parturition (7–15 d before the projected calving date; $n = 7$) or twinning ($n = 3$).

Table 1: Ingredients (%) and nutritional values of prepartal and postpartal rations (DM basis)*

Item	Prepartal Diet	Postpartal Diet
Corn silage (%)	32	27
Barley hay (%)	35	2
Clover (%)	0	19
Concentrated feed* (%)	33	52
Nutritive Value		
Dry matter (%)	56	66
Metabolizable energy (Mcal/kg)	1.90	2.18
Crude protein (CP, %)	11.3	16.5
Ruminally degradable protein (% CP)	64.0	61.7
Ruminally un-degradable protein (% CP)	36.0	38.3
Neutral detergent fiber (%)	50.4	38.4
Acid detergent fiber (%)	35.0	27.5
Starch (%)	14.5	21.8
Crude ash (%)	9.4	9.0
Crude fat (%)	2.0	2.9

* Nutrient composition values represent the basal TMR and exclude the top-dressed wheat bran and anionic supplement. During the prepartal period the animals in the experimental group received 1 kg/head/day of the anionic product (55 g of each of NH_4Cl , CaCl_2 and MgSO_4 per day (total 165 g/day), wheat bran, flaked corn and molasses, calculated DCAD: -3085 mEq/kg DM). The DCAD values of the control and treatment TMRs were estimated as +195 and -40 mEq/kg DM, respectively, according to the following equation (NRC, 2001): $\text{DCAD} = (\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{S}^{2-})$, (NRC, 2001).

Animals were fed ad libitum to yield 5 % orts once daily at 09:00 during the prepartum period and twice at 06:00 and 16:00 hrs during the postpartum period. All cows had free access to water throughout the experimental period.

Sample collection, laboratory analysis

Feed offered and refused were weighed daily before feeding for both groups to determine actual DMI throughout the experiment. The prepartum and postpartum TMR were sampled monthly and analyzed for crude protein, neutral detergent fiber, acid detergent fiber, ether extract, and ash by Nutrilab Analytical Services (Konya, Turkey). The nutrient compositions of prepartum and postpartum diets are shown in Table 1.

Cows were milked twice daily the first three days after calving and then milked thrice daily using an automatic dairy system (Alpro 6.60, DeLaval, Tumba, Sweden). Milk samples from three consecutive milkings on d 7 and d 14

were obtained, blended, and stored at -20°C until laboratory analysis for protein and fat concentration at the Ataturk University Food Engineering Laboratory.

Individual urine samples were collected on days -23, -14, and -7 relative to the expected calving date, in the morning, by manual vulvar stimulation. Determination of urine pH was performed within 15 min using a portable pH meter (Hanna HI83141, Sigma-Aldrich, St. Louis, MO).

About 10 ml of blood samples were taken from a coccygeal vein before morning feeding on d 23, 14 and 7 prior to expected calving day, and on d 1, 2, 3, 4, 5, 6, 7, 8, 15, and 28 after calving. The blood samples were centrifuged at 2800 *g* for 10 min and the sera were stored at -20°C until analyses for metabolic profile parameters (Ca, phosphorus (P), magnesium (Mg), serum urea-nitrogen (SUN), total protein (TP), albumin, glucose, cholesterol, aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), non-esterified fatty acids (NEFA), beta-hydroxy butyrate (BHBA) using spectrophotometry at the MG Institute (Konya, Turkey). Serum PTH concentrations were determined using a bovine-specific ELISA kit (Kit No: 11.192, BT LAB, Shanghai, China) at the Department of Biochemistry, Faculty of Veterinary Medicine, Ataturk University.

Milk fever was diagnosed based on clinical signs including recumbency and inability to stand during the early postpartum period. Retained placenta was defined as failure to expel the fetal membranes within 24 h after calving. Udder edema was assessed based on visible swelling of the udder and teat congestion. Mastitis was diagnosed according to abnormal milk appearance, clinical udder findings, and California Mastitis Test (CMT) results. Metritis was diagnosed based on abnormal vaginal discharge accompanied by fetid odor and increased rectal temperature during the postpartum period. After the first milking, colostrum density was measured using a hand-held refractometer (VBR 32, Chuyi Co., ChangSha, China).

Statistical analysis

Performance and metabolic profile parameters were subjected to the Proc. Mixed Procedure as two-way ANOVA in a complete randomized block design, separately for the prepartum and postpartum periods. Cow within dietary treatment was random term. The linear

model included block (breed and parity) and dietary treatment as well as dietary treatment by time interaction as fixed factors as well as time as repeated measures. The linear model was as follows: $Y = \mu + B_i + TRT_j + (B \times TRT)_{ij} + cow_k(TRT_j) + T_l + (B \times T)_{il} + (TRT \times T)_{jl} + e_{ijkl}$, where μ = population mean, B = i^{th} block, TRT = j^{th} dietary treatment, T = l^{th} day relative to parturition, and e = split plot error. The model included the first autoregressive (AR) covariance structure to yield lowest Aaike's value. Health parameters were analyzed using chi-square method. p value less than 0.05 using the Fisher's exact test option was considered significant and those between 0.05 and 0.10 were considered tendency due to small sample size (SAS Version 9.0, Statistical Analysis System, NC).

RESULTS

Performance parameters

Prepartum anionic salt supplementation resulted in a significant reduction in DMI compared with the control diet (11.25 vs. 11.78 kg/d; $P < 0.0001$; Table 2, Fig. 1). However, DMI during the prepartum period was not affected by breed or by the breed $\times$ treatment interaction. In the postpartum period, DMI tended to be higher in cows receiving the anionic diet compared with controls (25.78 vs. 25.64 kg/d; $P = 0.0530$). Holstein cows had higher postpartum DMI than Simmental cows (25.82 vs. 25.64 kg/d; $P = 0.0282$). A significant breed $\times$ treatment interaction was also observed postpartum ($P = 0.0107$), with DMI decreasing in Holstein cows but increasing in Simmental cows following anionic salt supplementation relative to their respective controls.

Prepartum anionic salt supplementation had no overall effect on postpartum milk yield. As expected, Holstein cows produced more milk than Simmental cows (31.32 vs. 28.48 L/d; $P < 0.0001$; Table 2, Fig. 2). However, the response to anionic salt supplementation differed by breed, with milk yield increasing in Holstein cows but decreasing in Simmental cows ($P < 0.0001$). Among milk components, only milk protein concentration was significantly affected by treatment. Cows supplemented with anionic salts had lower milk protein percentages than control cows (3.05 vs. 3.67 %; $P = 0.0278$; Table 2). Milk fat percentage and fat-to-protein ratio were not affected by treatment, breed, or their interaction.

Table 2: Effect of prepartal anionic salt feeding on performance of peripartum Holstein and Simmental Cows

Item	Pre-DMI (kg/d)	Post-DMI (kg/d) ^{1,2}	Milk Yield (L/d) ¹	Milk Fat (%)	Milk Protein (%)	Fat:Protein
Treatment effect						
Control	11.78 $\pm$ 0.03	25.64 $\pm$ 0.17	30.04 $\pm$ 0.60	4.13 $\pm$ 0.15	3.67 $\pm$ 0.21	1.21 $\pm$ 0.08
Anionic Salt	11.25 $\pm$ 0.04	25.78 $\pm$ 0.16	29.46 $\pm$ 0.51	4.00 $\pm$ 0.07	3.05 $\pm$ 0.18	1.41 $\pm$ 0.09
Breed effect						
Holstein	11.48 $\pm$ 0.04	25.82 $\pm$ 0.18	31.32 $\pm$ 0.68	4.00 $\pm$ 0.07	3.48 $\pm$ 0.23	1.25 $\pm$ 0.08
Simmental	11.45 $\pm$ 0.04	25.64 $\pm$ 0.16	28.48 $\pm$ 0.44	4.12 $\pm$ 0.14	3.27 $\pm$ 0.18	1.36 $\pm$ 0.09
Factorial arrangement						
Control, Holstein	11.72 $\pm$ 0.04	25.86 $\pm$ 0.23	29.62 $\pm$ 0.91	4.09 $\pm$ 0.10	3.88 $\pm$ 0.31	1.15 $\pm$ 0.12
Control, Simmental	11.83 $\pm$ 0.05	25.39 $\pm$ 0.26	30.54 $\pm$ 0.75	4.18 $\pm$ 0.31	3.41 $\pm$ 0.26	1.28 $\pm$ 0.12
Anionic Salt, Holstein	11.26 $\pm$ 0.06	25.76 $\pm$ 0.28	33.58 $\pm$ 0.98	3.87 $\pm$ 0.04	2.86 $\pm$ 0.21	1.40 $\pm$ 0.10
Anionic Salt, Simmental	11.24 $\pm$ 0.05	25.79 $\pm$ 0.20	27.29 $\pm$ 0.52	4.08 $\pm$ 0.10	3.16 $\pm$ 0.25	1.41 $\pm$ 0.13
P-value						
Treatment	0.0001	0.0530	0.2950	0.3445	0.0278	0.1210
Breed	0.3626	0.0282	0.0001	0.3908	0.7588	0.5739
Treatment $\times$ Breed	0.1802	0.0107	0.0001	0.7230	0.1701	0.6498

*Values are presented as least square means $\pm$ SEM. Control, Holstein (n = 6); Control, Simmental (n = 5); Anionic Salt, Holstein (n = 4); Anionic Salt, Simmental (n = 7). ¹Time effect, $P < 0.0001$. ²Treatment $\times$ Time interaction effect, $P = 0.0626$.

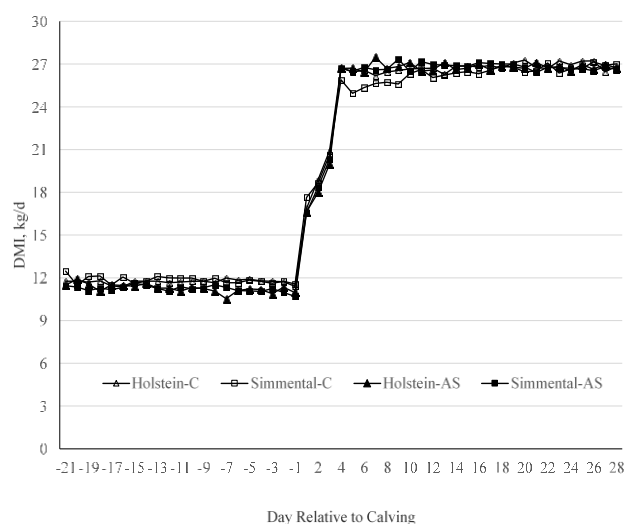


Fig. 1: DMI in response to prepartal anionic salt supplementation. Pooled SEM was 0.201 and 0.327 for during prepartum and postpartum, respectively. Control, Holstein (n = 6); Control, Simmental (n = 5); Anionic Salt, Holstein (n = 4); Anionic Salt, Simmental (n = 7).

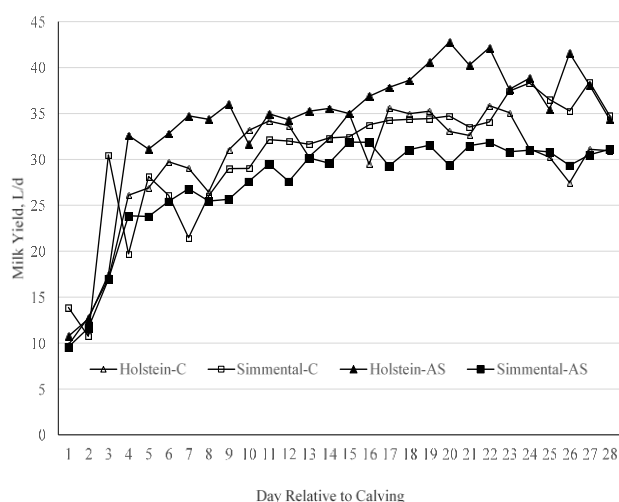


Fig. 2: Milk yield in response to prepartal anionic salt supplementation. Pooled SEM was 2.761. Values are presented as least square means $\pm$ SEM. Control, Holstein (n = 6); Control, Simmental (n = 5); Anionic Salt, Holstein (n = 4); Anionic Salt, Simmental (n = 7).

Peripartum metabolic profile

Prepartum anionic salt supplementation significantly reduced urine pH compared with the control group (7.27 vs. 8.01; $P < 0.0001$; Table 3). Simmental cows had slightly lower urine pH values than Holstein cows (7.58 vs. 7.67; $P = 0.0456$) and tended to exhibit a greater reduction in urine pH over time in response to anionic supplementation ($P = 0.0715$). As calving approached, urine pH in cows receiving the anionic diet declined to 6.88, whereas it remained at 7.98 in the control group ($P = 0.0254$; Fig. 3).

Prepartum anionic salt supplementation did not significantly affect serum PTH, Ca, P, or Mg concentrations (Table 3). Serum PTH concentrations were similar between Holstein and Simmental cows. However, Holstein cows had higher serum Ca (9.30 vs. 8.82 mg/dL; $P = 0.0008$) and Mg (2.14 vs. 1.99 mg/dL; $P = 0.0402$) concentrations and tended to have lower serum P concentrations than Simmental cows (6.14 vs. 6.47 mg/dL; $P = 0.0675$).

During the postpartum period, only serum Mg concentrations were affected by treatment, with higher values observed in cows receiving anionic salt supplementation (2.12 vs. 1.97 mg/dL; $P < 0.0002$; Table 4, Fig. 4). Holstein cows had lower serum PTH (11.42 vs. 12.97 pg/ml; $P = 0.0499$), Ca (8.58 vs. 8.42 mg/dL; $P = 0.0287$), and P (5.14 vs. 5.87 mg/dL; $P < 0.0001$) concentrations than Simmental cows postpartum. A significant treatment $\times$ breed interaction was observed for serum Ca concentrations ($P < 0.0001$), with Ca levels decreasing in Holstein cows but increasing in Simmental cows in response to prepartum anionic salt supplementation. No significant treatment $\times$ breed interactions were observed for serum PTH, P, or Mg concentrations.

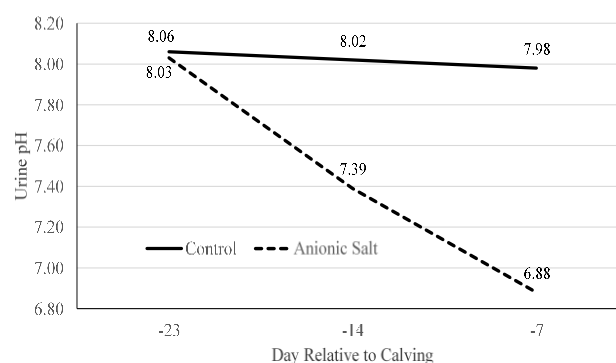


Fig. 3: Urine pH of cows in response to prepartal anionic salt supplementation. Treatment $\times$ Time interaction effect, $P = 0.0254$. Control (n = 11), Anionic Salt (n = 11).

As shown in Table 5 and 6, anionic salt supplementation increased serum urea nitrogen (SUN) and total protein (TP) concentrations during both the prepartum and postpartum periods. During the prepartum period, SUN (13.8 vs. 11.8 mg/dL; $P = 0.0107$) and TP (8.11 vs. 7.53 g/dL; $P = 0.0151$) concentrations were significantly higher in cows receiving anionic salt supplementation. No significant treatment effects were observed for albumin, glucose, cholesterol, AST, or GGT concentrations. Likewise, BHBA and NEFA concentrations were not affected by treatment (Fig. 5). Holstein cows had lower SUN and TP concentrations but higher albumin concentrations than Simmental cows. A tendency for a treatment $\times$ breed interaction was observed for glucose concentrations ($P = 0.0688$), with a greater decrease detected in Simmental cows than in Holstein cows following anionic salt supplementation. Serum GGT concentrations also tended to show a treatment $\times$ breed interaction ($P = 0.0588$), decreasing in Holstein cows but increasing in Simmental cows.

During the postpartum period, SUN (17.9 vs. 15.1 mg/dL; $P < 0.0001$) and TP (8.36 vs. 7.66 g/dL; $P < 0.0001$) concentrations remained higher in cows that had received prepartum anionic salt supplementation. The increase in SUN concentrations was more pronounced in Holstein cows than in Simmental cows ($P < 0.0001$).

Table 3: Effect of prepartal anionic salt feeding on urine pH and blood parathyroid hormone and macro mineral concentrations during the prepartum period*

Item	Urine pH ^{1,2,3,4}	PTH (pg/ml)	Ca (mg/dL)	P (mg/dL)	Mg (mg/dL)
Treatment effect					
Control	8.01 ± 0.03	6.93 ± 1.29	8.96 ± 0.09	6.18 ± 0.11	2.04 ± 0.05
Anionic Salt	7.27 ± 0.11	9.90 ± 1.92	9.03 ± 0.08	6.43 ± 0.12	2.08 ± 0.06
Breed effect					
Holstein	7.67 ± 0.12	6.79 ± 1.82	9.20 ± 0.06	6.14 ± 0.10	2.14 ± 0.06
Simmental	7.58 ± 0.10	9.78 ± 1.49	8.82 ± 0.09	6.47 ± 0.12	1.99 ± 0.04
Factorial arrangement					
Control, Holstein	7.98 ± 0.04	6.03 ± 1.92	9.11 ± 0.07	6.05 ± 0.14	2.08 ± 0.08
Control, Simmental	8.05 ± 0.03	8.02 ± 1.76	8.78 ± 0.16	6.34 ± 0.16	2.00 ± 0.06
Anionic Salt, Holstein	7.30 ± 0.22	7.93 ± 3.87	9.30 ± 0.08	6.23 ± 0.13	2.22 ± 0.10
Anionic Salt, Simmental	7.25 ± 0.13	11.03 ± 2.20	8.84 ± 0.11	6.56 ± 0.17	1.98 ± 0.06
P-value					
Treatment	0.0001	0.3230	0.2626	0.2240	0.4311
Breed	0.0456	0.3060	0.0008	0.0675	0.0402
Treatment × Breed	0.0715	0.5881	0.8201	0.5881	0.2868

*Values are presented as least square means ± SEM. Control, Holstein (n = 6); Control, Simmental (n = 5); Anionic Salt, Holstein (n = 4); Anionic Salt, Simmental (n = 7). PTH = parathyroid hormone. ¹Time effect, P = 0.0055. ²Treatment x Time interaction effect, P = 0.0254. ³Breed x Time interaction effect, P = 0.0717. ⁴Treatment x Breed x Time interaction effect, P = 0.0164.

Table 4: Effect of prepartal anionic salt feeding on blood parathyroid hormone and macro mineral concentrations during the postpartum period

Item	PTH (pg/ml)	Ca (mg/dL) ¹	P (mg/dL) ¹	Mg (mg/dL) ¹
Treatment effect				
Control	11.82 ± 0.51	8.49 ± 0.06	5.44 ± 0.12	1.97 ± 0.03
Anionic Salt	12.72 ± 0.51	8.50 ± 0.06	5.64 ± 0.12	2.12 ± 0.03
Breed effect				
Holstein	11.42 ± 0.46	8.58 ± 0.06	5.14 ± 0.12	2.06 ± 0.03
Simmental	12.97 ± 0.53	8.42 ± 0.06	5.87 ± 0.11	2.04 ± 0.03
Factorial arrangement				
Control, Holstein	10.80 ± 0.62	8.72 ± 0.08	5.18 ± 0.14	2.01 ± 0.04
Control, Simmental	13.04 ± 0.83	8.22 ± 0.08	5.75 ± 0.18	1.92 ± 0.04
Anionic Salt, Holstein	12.36 ± 0.66	8.39 ± 0.10	5.09 ± 0.20	2.12 ± 0.05
Anionic Salt, Simmental	12.93 ± 0.71	8.56 ± 0.08	5.96 ± 0.13	2.12 ± 0.03
P-value				
Treatment	0.5750	0.7045	0.4300	0.0002
Breed	0.0499	0.0287	0.0001	0.3164
Treatment × Breed	0.5881	0.0001	0.3488	0.2196

* Values are presented as least square means ± SEM Control, Holstein (n = 6); Control, Simmental (n = 5); Anionic Salt, Holstein (n = 4); Anionic Salt, Simmental (n = 7). PTH = parathyroid hormone. ¹Day Effect, P < 0.0001.

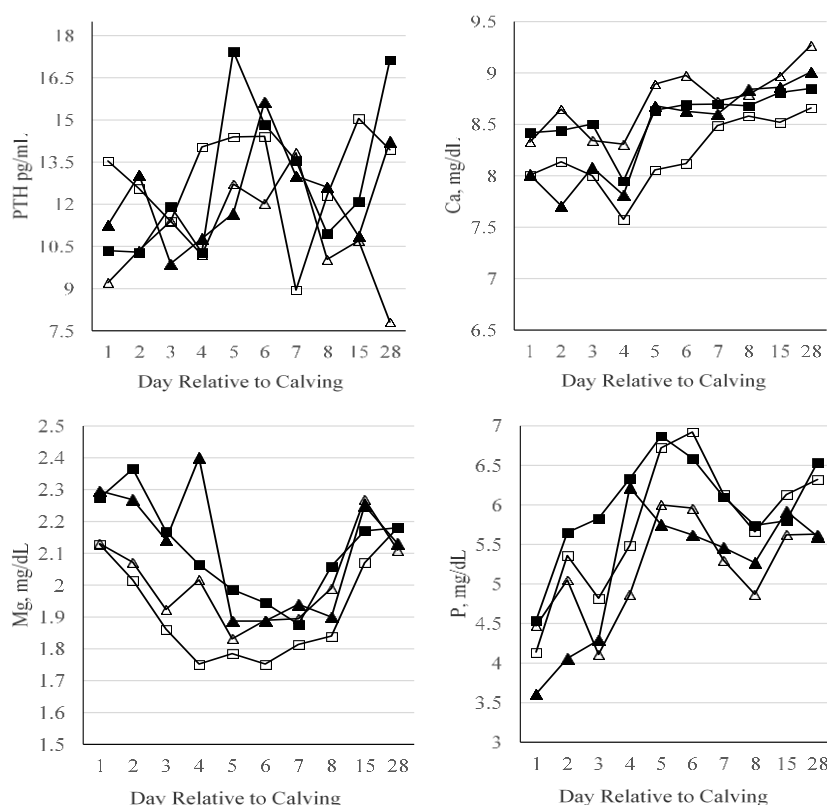


Fig. 4: Serum PTH and macro mineral (Ca, Mg and P) concentrations of cows in response to prepartal anionic salt supplementation. (---Δ---, Holstein control; ---▲---, Holstein anionic salt; ---□---, Simmental control; ---■---, Simmental anionic salt). Treatment x Time interaction effect on serum Ca, P < 0.0001. Pooled SEM was 2.13, 0.24, 0.11, and 0.43 for serum PTH, Ca, Mg, and P concentrations, respectively. Control, Holstein (n = 6); Control, Simmental (n = 5); Anionic Salt, Holstein (n = 4); Anionic Salt, Simmental (n = 7).

Table 5: Effect of prepartal anionic salt feeding on blood metabolic profile during the prepartum period

Item	SUN (mg/dL)	TP (g/dL)	Alb (g/dL)	Glu (mg/dL)	Chol (mg/dL)	BHBA (mmol/L)	NEFA (mmol/L)	AST (U/L)	GGT (U/L)
Treatment effect									
Control	11.8 ± 0.6	7.53 ± 0.1	3.48 ± 0.04	60.9 ± 1.6	95.0 ± 4.5	0.42 ± 0.06	0.34 ± 0.12	84.0 ± 2.8	21.2 ± 1.0
Anionic Salt	13.8 ± 0.4	8.11 ± 0.2	3.32 ± 0.08	58.0 ± 1.4	90.2 ± 3.8	0.35 ± 0.05	0.16 ± 0.02	78.6 ± 1.8	19.9 ± 0.9
Breed effect									
Holstein	11.3 ± 0.5	7.54 ± 0.1	3.51 ± 0.05	59.4 ± 1.8	96.0 ± 3.7	0.46 ± 0.06	0.33 ± 0.13	82.9 ± 2.0	21.5 ± 1.2
Simmental	14.2 ± 0.4	8.11 ± 0.2	3.30 ± 0.07	59.3 ± 1.3	89.4 ± 4.4	0.31 ± 0.04	0.22 ± 0.06	80.5 ± 2.6	19.6 ± 0.8
Factorial arrangement									
Control, Holstein	10.3 ± 0.5	7.35 ± 0.2	3.55 ± 0.04	60.6 ± 2.8	99.0 ± 6.1	0.47 ± 0.08	0.39 ± 0.18	85.9 ± 2.8	23.2 ± 1.5
Control, Simmental	13.7 ± 0.7	7.74 ± 0.2	3.41 ± 0.07	61.3 ± 1.4	90.4 ± 6.6	0.33 ± 0.05	0.26 ± 0.09	84.0 ± 5.3	18.8 ± 0.9
Anionic Salt, Holstein	12.5 ± 0.8	7.76 ± 0.2	3.46 ± 0.10	58.0 ± 2.1	92.5 ± 3.6	0.43 ± 0.02	0.17 ± 0.03	79.3 ± 2.5	19.6 ± 1.7
Anionic Salt, Simmental	14.6 ± 0.4	8.35 ± 0.2	3.23 ± 0.10	58.0 ± 2.0	88.7 ± 6.0	0.28 ± 0.08	0.15 ± 0.03	78.2 ± 2.6	20.2 ± 1.1
P-value									
Treatment	0.0107	0.0151	0.1430	0.1867	0.4915	0.6071	0.3859	0.0722	0.3936
Breed	0.0001	0.0192	0.0365	0.8636	0.3019	0.1302	0.7054	0.6496	0.1611
Treatment × Breed	0.2864	0.6916	0.6397	0.0688	0.8636	0.6134	0.7829	0.9109	0.0588

*Values are presented as least square means ± SEM. Control, Holstein (n = 6); Control, Simmental (n = 5); Anionic Salt, Holstein (n = 4); Anionic Salt, Simmental (n = 7). SUN = serum urea-nitrogen; TP = total protein; Alb = albumin; Glu = glucose; Chol = cholesterol; BHBA = beta-hydroxy butyrate; NEFA = non-esterified fatty acids; AST = aspartate aminotransferase; GGT = gamma-glutamyl transferase.

Table 6: Effect of prepartal anionic salt feeding on blood metabolic profile during the postpartum period (LSM ± SEM)

Item	SUN (mg/dL) ¹	TP (g/dL) ²	Alb (g/dL) ³	Glu (mg/dL) ²	Chol (mg/dL) ²	BHBA (mmol/L) ⁴	NEFA (mmol/L) ⁵	AST (U/L) ²	GGT (U/L)
Treatment effect									
Control	15.1 ± 0.4	7.66 ± 0.08	3.32 ± 0.03	51.6 ± 1.7	83.0 ± 2.9	0.59 ± 0.05	0.58 ± 0.05	109 ± 2	22.8 ± 0.6
Anionic Salt	17.9 ± 0.4	8.36 ± 0.09	3.27 ± 0.03	48.4 ± 1.6	80.0 ± 2.6	0.59 ± 0.06	0.49 ± 0.05	117 ± 5	22.1 ± 0.5
Breed effect									
Holstein	16.1 ± 0.5	7.75 ± 0.08	3.31 ± 0.03	46.8 ± 1.5	83.0 ± 2.7	0.64 ± 0.05	0.63 ± 0.06	114 ± 5	23.3 ± 0.6
Simmental	16.9 ± 0.3	8.25 ± 0.10	3.28 ± 0.03	52.6 ± 1.8	80.2 ± 2.7	0.55 ± 0.06	0.46 ± 0.04	112 ± 3	21.8 ± 0.5
Factorial arrangement									
Control, Holstein	13.9 ± 0.47	7.55 ± 0.09	3.33 ± 0.04	49.1 ± 2.0	84.1 ± 3.7	0.67 ± 0.07	0.65 ± 0.08	104 ± 3	24.0 ± 0.8
Control, Simmental	16.5 ± 0.5	7.79 ± 0.14	3.30 ± 0.04	54.5 ± 2.8	81.7 ± 4.6	0.50 ± 0.06	0.51 ± 0.05	114 ± 3	21.4 ± 0.7
Anionic Salt, Holstein	19.2 ± 0.7	8.02 ± 0.12	3.27 ± 0.04	43.6 ± 2.2	81.4 ± 4.0	0.61 ± 0.06	0.61 ± 0.09	127 ± 11	22.2 ± 1.0
Anionic Salt, Simmental	17.2 ± 0.4	8.56 ± 0.13	3.27 ± 0.04	51.2 ± 2.2	79.1 ± 3.4	0.58 ± 0.09	0.42 ± 0.05	111 ± 5	22.1 ± 0.6
P-value									
Treatment	0.0001	0.0001	0.2853	0.0529	0.2858	0.9685	0.3338	0.0806	0.3793
Breed	0.8114	0.0014	0.4050	0.0088	0.0552	0.4293	0.0798	0.5010	0.2044
Treatment × Breed	0.0001	0.1270	0.8115	0.5169	0.7890	0.3804	0.5500	0.0208	0.0819

* Values are presented as least square means ± SEM. Control, Holstein (n = 6); Control, Simmental (n = 5); Anionic Salt, Holstein (n = 4); Anionic Salt, Simmental (n = 7). SUN = serum urea-nitrogen; TP = total protein; Alb = albumin; Glu = glucose; Chol = cholesterol; BHBA = beta-hydroxy butyrate; NEFA = non-esterified fatty acids; AST = aspartate aminotransferase; GGT = gamma-glutamyl transferase; ¹Day Effect, P = 0.0020. ²Day Effect, P < 0.0001. ³Day Effect, P = 0.0082. ⁴Day Effect, P = 0.0148. ⁵Day Effect, P = 0.0129.

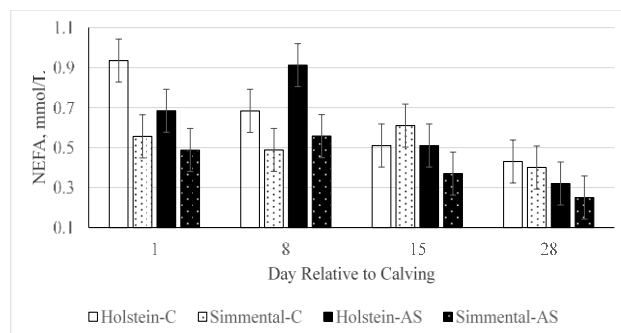
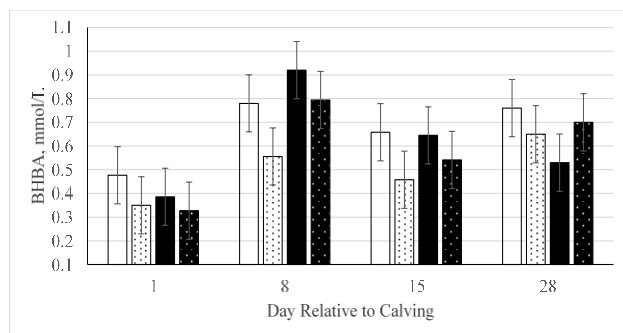


Fig. 5: Serum BHBA and NEFA concentrations of cows in response to prepartal anionic salt supplementation. Control, Holstein (n = 6); Control, Simmental (n = 5); Anionic Salt, Holstein (n = 4); Anionic Salt, Simmental (n = 7).

Postpartum health and colostrum quality

Due to limited sample size, the effect of prepartum anionic salt feeding on postpartum health outcomes should be interpreted cautiously. No cases of milk fever were recorded. The anionic group showed a trend toward lower incidence of mastitis (P = 0.0624; Table 7) and had fewer

cases of retained placenta.

Colostrum quality, as measured by Brix refractometer, was significantly improved by prepartum anionic salt supplementation (26.23 vs. 23.00%; P = 0.0059; Table 8). No significant effects of breed or treatment-by-breed interaction were observed.

Table 7: Effect of prepartal anionic salt feeding on health status during the postpartum period

Disease	Control	Anionic Salt
Mastitis +	3 (27.27%)	0 (0%)
Mastitis -	8 (72.73%)	11 (100%)
Statistics	$\chi^2 = 3.4737$ and $P = 0.0624$	
Edema +	1 (9.09%)	0 (0%)
Edema -	10 (90.91%)	11 (100%)
Statistics	$\chi^2 = 1.0476$ and $P = 0.3061$	
Metritis +	1 (9.09%)	0 (0%)
Metritis -	10 (90.91%)	11 (100%)
Statistics	$\chi^2 = 1.0476$ and $P = 0.3061$	
Retained placenta +	4 (36.36%)	1 (9.09%)
Retained placenta -	7 (63.64%)	10 (90.91%)
Statistics	$\chi^2 = 2.3294$ and $P = 0.1269$	

*Control, Holstein (n = 6); Control, Simmental (n = 5); Anionic Salt, Holstein (n = 4); Anionic Salt, Simmental (n = 7).

Table 8: Effect of prepartal anionic salt feeding on colostrum quality

Treatment	Breed	Colostrum Quality (Brix, %)
Control		23.00 ± 0.67
Anionic Salt		26.23 ± 0.71
	Holstein	24.27 ± 0.59
	Simmental	25.15 ± 0.97
Control	Holstein	23.50 ± 0.92
	Simmental	22.40 ± 1.03
Anionic Salt	Holstein	25.20 ± 0.49
	Simmental	26.88 ± 1.08
ANOVA	P > F	
Treatment		0.0059
Breed		0.7774
Treatment x Breed		0.1818

* Values are presented as least square means ± SEM. Control, Holstein (n = 6); Control, Simmental (n = 5); Anionic Salt, Holstein (n = 4); Anionic Salt, Simmental (n = 7).

DISCUSSION

Subclinical milk fever as a primary postpartum disease triggers the development of secondary postpartum diseases, such as dystocia, retained placenta, metritis, mastitis, displaced abomasum, and ketosis. This experiment was therefore focused on the elimination of hypocalcemia, while not interfering with appetite and avoiding hypomagnesemia at parturition and early postpartum via feeding prepartal anionic salt. Anionic salt supplementation during the prepartum period led to a modest but statistically significant reduction in DMI, consistent with previous findings attributing this effect to reduced palatability (Hu and Murphy 2004; Vagnoni and Oetzel 1998). However, the 4.4% decrease observed in this study did not adversely affect postpartum performance, supporting Oetzel (2015) suggestion that moderate reductions in prepartum DMI are tolerable. Postpartum DMI tended to be higher in the anionic group, aligning with findings by Degroot et al. (2010) and Weich et al. (2013). These results suggest that pelleting anionic salts with molasses may mitigate negative effects on feed intake.

No overall treatment effect was observed on milk yield, though breed-specific differences were evident. Holstein cows produced more milk than Simmental cows, as expected, but responded differently to anionic feeding. While milk yield increased in Holsteins receiving anionic salts, it decreased in Simmentals. This divergent response

does not appear to be directly related to DMI, as Simmental cows actually showed increased DMI postpartum. Rather, it may reflect inherent breed differences in nutrient partitioning or metabolic efficiency during early lactation.

Anionic salt supplementation was associated with lower milk protein concentration during early lactation. Similar reductions in milk protein content have also been reported in cows fed low-DCAD diets (Leno et al. 2017; Rajaeerad et al. 2023). Some of these studies observed more pronounced urinary acidification (urine pH < 6.0), suggesting that the degree of dietary acidification may influence milk protein responses (Rajaeerad et al. 2023). Although the underlying mechanism remains unclear, alterations in acid-base balance, parity, ruminal fermentation characteristics, nutrient partitioning, or amino acid utilization during the transition period may contribute to these variable responses (Lean et al. 2019).

Urine pH is a convenient and practical indicator for evaluating systemic acid-base status and monitoring the efficacy of anionic salt supplementation. Numerous studies have shown that feeding anionic salts significantly reduces urine pH (Ramos-Nieves et al. 2009; Glosston et al. 2020; Goff et al. 2020). In the present study, Simmental cows exhibited a greater reduction in urine pH and a more favorable increase in serum calcium compared to Holstein cows, suggesting a stronger physiological response to dietary acidification. These breed-related differences may be attributed to variations in acid-base regulation, PTH sensitivity, or ruminal buffering capacity. Accordingly, dual-purpose breeds such as Simmental may require less aggressive acidification to achieve optimal calcium homeostasis. In contrast, the level of acidification applied in this study appeared insufficient for Holstein cows, as their urine pH remained slightly above the recommended target range of 6.2–6.8 for effective prevention of subclinical hypocalcemia (Goff et al. 2007).

While the effects of prepartum anionic feeding on phosphorus and magnesium status remain inconsistent in the literature, several studies have highlighted the close physiological relationship among calcium, phosphorus, and magnesium metabolism during the transition period (Lean et al. 2006; Silva et al. 2023). Magnesium is particularly important for calcium homeostasis because it plays a central role in PTH secretion and tissue responsiveness to PTH (Degaris and Lean 2008; Goff 2018). In the present study, elevated serum magnesium concentrations in cows receiving anionic supplementation may have been associated with the MgSO_4 content of the supplement, consistent with previous reports suggesting that increased serum magnesium concentrations in cows fed acidogenic diets may partly reflect dietary magnesium supply rather than a direct physiological effect of DCAD itself (Goff et al. 1991, Lean et al. 2006; Li et al. 2008). However, previous studies evaluating the influence of DCAD on magnesium and phosphorus concentrations have reported inconsistent findings (Shahzad and Sarwar 2008; Razzaghi et al. 2012). In addition to its physiological role, MgSO_4 was selected because of its relatively better palatability compared with several other anionic salts, which may have helped minimize reductions in DMI.

Since AST is not liver-specific in cattle and may also reflect muscular or metabolic adaptation processes, these findings should be interpreted cautiously (Campos-Gaona et al. 2024). Nevertheless, increased AST activity during the transition period has also been associated with altered hepatic metabolic load and lipid mobilization (Stojevic et al. 2005; Campos-Gaona et al. 2024). In the present study, however, the absence of marked differences in BHBA, NEFA, and GGT concentrations weakens the interpretation of clinically relevant hepatic dysfunction and suggests that the observed AST response more likely reflected subtle breed-related metabolic variation.

Serum concentrations of SUN and total protein were higher in cows that received anionic salt supplementation during the prepartum period. While the direct nitrogen contribution of ammonium chloride is unlikely to persist into the postpartum period, the elevated values may reflect residual effects of prepartum acidification on nitrogen metabolism or hepatic function. Although BHBA and NEFA levels were not significantly affected by treatment, serum glucose tended to be lower in the anionic group. Simmental cows had lower cholesterol and NEFA concentrations compared to Holsteins, which may suggest a slightly less pronounced negative energy balance. However, glucose levels did not differ significantly between breeds, indicating that energy status-related differences may be subtle or influenced by other metabolic factors. These findings point to potential breed-related variations in energy metabolism, but further investigation is needed to clarify these physiological distinctions.

Sample size in this experiment is too small to make inferences about postpartum health status and colostrum quality in response to prepartal anionic salt feeding. To make a clear conclusion a larger data set is needed.

Conclusion

Subclinical milk fever as a primary postpartum disease triggers the development of secondary postpartum diseases, such as dystocia, retained placenta, metritis, mastitis, displaced abomasum, and ketosis. This experiment was therefore focused on the elimination of hypocalcemia, while not interfering with appetite and avoiding hypomagnesemia at parturition and early postpartum via feeding prepartal anionic salt. Pelleting anionic salts with molasses reduced unpalatability as reflected by a low depression in DMI. In contrast to Holstein cows, Simmental cows demonstrated increased postpartum feed intake, lower urine pH during the late prepartum period, and higher blood calcium levels in early lactation in response to prepartal anionic salt feeding. These findings suggest that Simmental cows may be more responsive to dietary acidification strategies. This breed-specific variation highlights the potential value of developing tailored DCAD management approaches to optimize metabolic adaptation and performance across different genetic backgrounds. However, this experiment has major limitations. Small sample size as well as few research articles available on cross-cattle requires caution on our conclusion statement. Further research involving larger populations and graded levels of anionic salts is warranted to confirm these breed-dependent responses.

DECLARATIONS

Funding: This study was funded by the Ataturk University Scientific Research Projects Coordination Office as the part of PhD project (#: TDK-2022-10233).

Acknowledgement: The authors thank to Mr. Esref Sekerli for providing bran, corn, and molasses and allowing his production unit to develop pelleted product (Kahramanmaraş, Turkey). A very special thanks goes to Mr. Mehmet Oner, the owner of Dutpinar Dairy Farm (Malatya, Turkey) to execute animal experimentation. We also thank veterinary technician Ugur Yildirim helping in choiring animals and Asst. Prof. Mustafa Ileriturk for hormone analysis.

Conflict of Interest: The authors declare no conflict of interest.

Data Availability: The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Statement: Procedures applied to the animals have been approved by the Ataturk University Ethics Committee (2021, #75296309-050.01.04-E.2100037077).

Author's Contribution: US was responsible for data collection, sample processing, and initial drafting of the manuscript. AH conceptualized the study design, conducted the statistical analysis, and revised the manuscript critically for intellectual content. Both authors contributed to the interpretation of results and approved the final version of the manuscript for submission.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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