



Breed-Specific Variations in Blood Metabolites and Cortisol Reduction in Response to Organic Mineral Supplementation in Simmental and Holstein Calves

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ABSTRACT

Early life immunity and stress regulation are critical for the health and survival of newborn calves. Trace minerals like selenium, zinc, and chromium, especially when obtained from organic sources, play an important role in immune and defense systems and in stress physiology. This study examined the effects of supplementing Holstein and Simmental calves with a blend of organic minerals (chromium, selenium, and zinc) on immune and stress responses and blood metabolites. Twenty Holstein and 20 Simmental calves were divided into four groups in a 2 × 2 factorial arrangement, with breed as the first and organic mineral supplementation as the second factor. In addition to milk, each calf received a mixture of organic selenium, chromium, and organic zinc (0.5 g each) orally for 21 days after birth. Calves in the control group did not receive any organic mineral supplement. Blood samples were collected from the jugular vein of all calves seven times: on the day of birth, after 3 days, and then once per week until weaning after the completion of oral mineral supplementation. Serum alanine transaminase, aspartate transaminase, gamma glutamyl transferase, high-density lipoprotein, low-density lipoprotein, and total cholesterol concentrations were 27%, 19.1%, 21.2%, 15.5%, 12.5%, and 13% higher, respectively, in Simmental calves than in Holstein calves, during the first week after birth. The addition of an organic mineral mixture to drinking milk did not affect blood metabolites in either breed ($p>0.05$), except for a 35% decrease in serum cortisol levels in both breeds during week 3 ($p<0.021$). Overall, it was concluded that organic minerals, in addition to milk, may have protective potential against stress by reducing serum cortisol levels during periods of stress in newborn calves of both Simmental and Holstein breeds.

Keywords: calf nutrition; calf breed; immunity; organic mineral; stress

INTRODUCTION

The sustainability of cattle herds within breeding enterprises depends on the success of healthy calf breeding programs. The most crucial period for calf care and breeding is the first 24 hours after birth (Gundelach *et al.*, 2009). Immaturity of the immune system of newborn calves leads to the highest mortality rates during the initial 7 days of the postpartum period. With an immature immune system, the transition from a functionally monogastric to ruminant status involves several metabolic, physiological, and nutritional changes (Rajaei-Sharifabadi *et al.*, 2024).

Various risk factors are directly associated with herd management, such as group housing and immunoglobulin deficiency resulting from inadequate colostrum intake, which contribute to calf mortality (Barry *et al.*, 2019; Olson *et al.*, 1999; Svensson *et al.*, 2003). With excellent care and feeding practices, mortality rates can be at or below 5% (Wynn *et al.*, 2009). Consequently, colostrum has emerged as a unique food

source for minimizing health problems. Newborn calves lack developed defense mechanisms against diseases; however, colostrum feeding provides passive protection by supplying immunoglobulins (IgG) that are actively absorbed by the small intestine (Korhonen *et al.*, 2000; Salles *et al.*, 2025; Uyama *et al.*, 2022).

Trace minerals are important micronutrients that play various roles in physiological processes, including oxidative metabolism and optimal cellular function (Ogilvie *et al.*, 2023). Trace minerals such as selenium, chromium, and zinc are critical for immune function and overall metabolism (Arthington *et al.*, 2014; Shambhvi *et al.*, 2023; Willmore *et al.*, 2021). Insufficient intake of these essential trace minerals can lead to clinical or subclinical symptoms, depending on the degree of deficiency. The optimal quantity of trace minerals included in diets depends on their bioavailability and the presence of antagonistic substances that may decrease their bioavailability (Byrne & Murphy, 2022). Despite being present in low concentrations within an organism, trace minerals play

crucial roles in numerous physiological processes, including vitamin synthesis, hormone production, enzyme activity, regulation of cell osmotic pressure, collagen formation, tissue synthesis, oxygen transport, energy production, growth, fertility, and overall health. Failure to meet these needs can result in severe economic losses for animal producers owing to their animals' decreased health and productivity. Inorganic salts (oxides, sulfates, and carbonates) are commonly used to meet the trace mineral needs of animals; however, products of organic origin have also been used in recent years (Harvey *et al.*, 2021; Mousavi-Haghshenas *et al.*, 2022). Organic minerals are formed by combining trace minerals in an organic matrix, such as amino acids or polysaccharides, through covalent bonds (Harvey *et al.*, 2021; Ram *et al.*, 2021).

At an early age, calves have limited prenatal trace mineral reserves, an immature immune system, and low endogenous antioxidant capacity, and they exhibit rapid metabolic adaptation, leading to distinct oxidative and physiological stress, which renders them highly dependent on postnatal mineral supply. Selenium and zinc are critical for antioxidant defense and immune maturation, whereas the stress response is mediated by chromium through the modulation of cortisol secretion and immune function, collectively justifying targeted trace mineral supplementation in early-age calves (Ghorbani *et al.*, 2012; Teixeira *et al.*, 2014). Newborn calves fed adequately with colostrum supplemented with an organic source of trace minerals can show improved health and metabolic status. To our knowledge, no study has been conducted to evaluate the effects of organic minerals on immunoglobulin G, cortisol as a stress biomarker, and blood metabolites in newborn calves, and comparing Holstein and Simmental breeds. This limits our understanding of how breed-specific responses to organic minerals may influence early life immune competence and stress resilience. Therefore, the objective of this study was to analyze and compare the effects of organic mineral mixtures containing selenium, chromium, and zinc on the levels of immunoglobulin G, cortisol, and blood metabolites in newborn Holstein and Simmental calves.

MATERIALS AND METHODS

All experimental protocols used in this experiment were approved by the Animal Ethics Committee of Afyon Kocatepe University, Afyonkarahisar, Turkey (01-120420).

Animals

Forty Holstein calves, including 20 Holstein and 20 Simmental calves of similar body weight (39.5 ± 1 kg), were used for this study. All cows had received similar pre- and postpartum diets. After careful observation at birth, the calves were removed within 15-20 minutes post-calving. The body weights of the calves were recorded using a digital weighing balance before colostrum feeding. Calves were housed individually and were not allowed to suckle their dams, which were

milked within 45 minutes of calving. The calves received the colostrum from their respective dams for 4 days after birth, after which they were provided milk from a tank designated for the calves.

Treatments and Experimental Design

The Holstein and Simmental calves received one of the two dietary treatments in a completely randomized design in a 2×2 factorial arrangement, resulting in the following four experimental groups; 1) Holstein control group fed only milk, 2) Holstein mineral group fed organic mineral supplement with milk (0.5 g/day of each organic mineral; selenium, chromium, and zinc), 3) Simmental control group fed only milk, and 4) Simmental mineral group fed organic mineral supplement with milk (0.5 g/day of each organic mineral; selenium, chromium, and zinc). Mineral supplements were provided to the experimental groups during the first 4 days after mixing in colostrum, after which the supplementation was added to the morning milk just before feeding to the calves of the respective groups. Organic mineral supplementation in the treatment groups continued from birth to 21 days of age.

Sampling and Analysis

The colostrum quantity was measured and sampled at the first milking stage for the analysis of colostrum components using an automatic milk analyzer. Fat, protein, lactose, and somatic cell counts were measured daily in the colostrum and tank milk provided to the calves. Blood was drawn from the calves on the day of birth (day 0), Bovi-Sera was administered, and mineral mixtures were given with milk to the respective treatment groups. Blood samples were taken from the jugular vein seven times at 3-day intervals during oral administration and once per week until weaning after the oral supplementation treatment was completed. Additional blood samples were collected from the calves on days 14 and 21 after birth to determine immunoglobulin G levels. The blood samples were placed in tubes without anticoagulants, centrifuged at $3000 \times g$, and incubated at room temperature for 15 minutes to obtain sera. The sera were then stored at -20 °C until analysis. Analyses of alanine transaminase (ALT; Biolabo SA, Maizy, France), aspartate transaminase (AST; Biolabo SA, Maizy, France), gamma-glutamyl transferase (GGT; Biolabo SA, Maizy, France), urea, uric acid, total protein (Biolabo SA, Maizy, France), total cholesterol (Biolabo SA, Maizy, France), triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and glucose (Biolabo SA, Maizy, France) were performed on the serum samples using commercially available kits. Cortisol and immunoglobulin G analyses were conducted using commercial kits (Cusabio Inc., Wuhan, Hubei, China), and all other variables were assessed using commercial kits according to the manufacturer's instructions (Biolabo SA, Maizy, France) and a ChemWell-2910 automatic chemistry analyzer (Awareness Technology Inc., Martin Hwy, Palm City, USA).

Statistical Analyses

The data were analyzed using Kolmogorov-Smirnov tests, which did not confirm normal distribution. Considering the sample size, the Kruskal-Wallis H test was used to compare each parameter across different terms between the groups. Additionally, a Bonferroni-corrected Mann-Whitney U test was applied to avoid type-1 errors in determining the terms during which differences were observed between the groups. For time-dependent variables, the Friedman test was applied, and a Bonferroni-corrected Mann-Whitney U test was used to address type-1 error in terms where differences were noted between the groups. Except for the tests with Bonferroni correction, all tests resulted in significance levels of $p < 0.05$. Data analysis was conducted using PASW Statistics software (version 18.0), and the results are presented as means with standard error of the mean.

RESULTS

The composition of Holstein colostrum, Simmental colostrum, and tank milk differed (Table 1). Colostrum fat, protein, lactose, and somatic cell count (SCC) were higher in the colostrum of Simmental cows than in that of Holstein cows; the lowest values of fat, protein, lactose, and SCC were observed in tank milk samples ($p < 0.001$).

The effects of organic minerals on the blood metabolites of Holstein and Simmental calves were compared (Table 2). When comparing blood biochemical variables within the group over time, no differences were observed in serum urea, uric acid, triglyceride, or total protein levels ($p > 0.05$). However, time-dependent changes were observed in AST, ALT, GGT, LDL, HDL, cholesterol, and glucose levels ($p < 0.05$). Among the various treatment groups, differences were observed in AST, ALT, GGT, LDL, HDL, and cholesterol levels on various days ($p < 0.05$). However, organic mineral supplementation had no effect on serum urea, uric acid, glucose, triglyceride, or total protein concentrations ($p > 0.05$). Serum AST levels in Holstein calves at birth and during the first 9 days after birth were lower than those in Simmental calves ($p < 0.05$), irrespective of mineral supplementation ($p > 0.05$). Similarly, serum ALT levels were lower in Holstein calves than in Simmental calves on days 0 and 3 after birth ($p < 0.05$). Serum ALT levels remained higher in all groups until day 3 ($p < 0.05$) and did not change afterwards ($p > 0.05$). Serum GGT levels remained higher on various days postpartum ($p > 0.05$). Serum LDL levels were lower in Holstein calves than in Simmental calves on day 0 ($p < 0.05$). Serum chole-

sterol, HDL, and LDL levels increased during the first 21 days after birth in both Simmental and Holstein calves ($p < 0.05$). Serum glucose levels decreased gradually during the first 21 days after birth ($p < 0.05$).

Serum cortisol levels changed in a time-dependent manner in all calves (Tables 3 and 4). Serum cortisol levels decreased with increasing days of age in both Holstein and Simmental calves ($p < 0.05$). Although no effect of organic mineral supplementation was observed early postpartum, serum cortisol decreased significantly from days 15 to 21 in calves supplemented with organic minerals ($p < 0.05$). From 28 days of age, serum cortisol levels increased until 42 days postpartum and decreased afterwards ($p < 0.05$). However, no differences were observed in cortisol levels with regard to breed or mineral supplementation ($p > 0.05$).

Serum IgG concentrations were similar in Holstein and Simmental calves, irrespective of organic mineral supplementation (Table 5; $p < 0.05$). However, serum IgG concentrations decreased with increasing age in all calves ($p < 0.05$).

DISCUSSION

Serum levels of AST, ALT, and GGT provide indications of liver function. These three liver enzymes typically increase in the case of acute or chronic liver disease (Stojević *et al.*, 2005). Serum AST and GGT levels are increased in adult cows with subclinical diseases such as fatty liver syndrome and ketosis (Pinedo *et al.*, 2022; Steen, 2001). The AST activity in newborn calves ranges from 5 to 40 IU/L in the first few days after birth (Quezada-Tristán *et al.*, 2014). ALT and AST activities ranged from 1 to 5 IU/L and from 19 to 31 IU/L, respectively, in calves aged 7–27 days, with both levels increasing with age (Kaneko *et al.*, 2008). According to previous studies, serum ALT levels decreased in calves given zinc and methionine for 8 weeks after birth, but serum AST levels did not change (Ülger & Küçük, 2011). In other animal species, the addition of zinc to rations or an increase in zinc in the serum increases ALT and AST activity (Liu *et al.*, 2023; Wei *et al.*, 2019).

Serum total protein, urea, and uric acid levels in calves provide information on metabolic and growth-related activities. Pekcan *et al.* (2023) reported that liver diseases and conditions associated with infections, such as diarrhea and arthritis in newborn calves, did not change the blood total protein and uric acid levels, whereas urea levels may increase more than two-fold. According to their findings, the serum urea level in healthy calves was 17.73 ± 2.80 mg/dL, whereas in animals with arthritis and diarrhea, it increased to 41.54 ± 5.29 mg/dL.

Table 1. Milk and colostrum profiles of Simmental and Holstein dams used in the study

Breed	Variables			
	Fat (%)	Protein (%)	Lactose (%)	SCC (x103)
Simmental (colostrum)	6.9 ± 0.81	9.7 ± 1.42	3.5 ± 1.12	319 ± 23.1
Holstein (colostrum)	6.1 ± 0.64	9.2 ± 1.51	2.9 ± 1.34	311 ± 20.8
Tank milk	4.1 ± 0.51	3.6 ± 1.57	5.4 ± 1.25	157 ± 18.2

Note: SCC, Somatic cell count.

Table 2. Blood metabolites of Holstein and Simmental calves (first 21 days) in response to organic minerals supplementation

Variables	Day	Treatments				P		
		Holstein		Simmental		Treat	Mineral	Breed
		Control	Mineral	Control	Mineral			
AST (U/L)	0	38.5 ± 5.78 ^{Aa}	39.1 ± 4.89 ^{Aa}	50.1 ± 3.12 ^{Ba}	51.7 ± 5.07 ^{Ba}	0.012	0.003	0.015
	3	40.1 ± 4.23 ^{Aa}	41.7 ± 3.61 ^{Aa}	51.0 ± 4.40 ^{Ba}	51.1 ± 5.12 ^{Ba}	0.024	0.043	0.001
	6	41.3 ± 3.78 ^{Aa}	42.5 ± 4.22 ^{Aa}	53.1 ± 5.04 ^{Ba}	51.9 ± 0.05 ^{Ba}	0.011	0.766	0.019
	9	38.5 ± 3.57 ^{Aa}	40.5 ± 5.09 ^{Aa}	53.7 ± 4.89 ^{Ba}	52.7 ± 3.38 ^{Ba}	0.041	0.017	0.047
	12	48.4 ± 6.12 ^b	46.2 ± 3.67 ^b	50.2 ± 4.38 ^b	51.1 ± 3.15 ^b	0.899	0.107	0.151
	15	52.1 ± 5.08 ^b	51.7 ± 3.32 ^b	52.7 ± 4.82 ^b	53.7 ± 5.38	0.856	0.321	0.138
	18	52.3 ± 4.19 ^b	53.7 ± 5.31 ^b	50.3 ± 4.42 ^b	51.4 ± 3.21 ^b	0.912	0.281	0.407
	21	54.0 ± 6.52 ^b	53.1 ± 4.43 ^b	54.4 ± 5.31 ^b	52.3 ± 4.56 ^b	0.954	0.710	0.519
	P	0.047	0.042	0.035	0.049			
	ALT (U/L)	0	10.6 ± 3.19 ^{Aa}	11.7 ± 3.38 ^{Aa}	14.3 ± 3.12 ^{Ba}	13.8 ± 3.42 ^{Ba}	0.041	0.003
3		11.1 ± 4.91 ^{Aa}	10.5 ± 3.18 ^{Aa}	14.6 ± 3.71 ^{Ba}	13.5 ± 5.02 ^{Ba}	0.048	0.073	0.683
6		12.4 ± 4.65 ^b	13.3 ± 4.23 ^b	13.3 ± 4.68 ^b	13.4 ± 3.78 ^b	0.072	0.039	0.866
9		14.1 ± 5.61 ^b	12.5 ± 4.19 ^b	14.4 ± 3.31 ^b	13.7 ± 5.02 ^b	0.081	0.255	0.299
12		16.1 ± 6.12 ^b	15.4 ± 5.60 ^b	13.7 ± 3.82 ^b	15.6 ± 5.10 ^b	0.086	0.535	0.861
15		16.2 ± 5.91 ^b	16.7 ± 4.01 ^b	15.9 ± 3.71 ^b	16.6 ± 3.45 ^b	0.091	0.053	0.139
18		15.5 ± 3.89 ^b	14.7 ± 4.33 ^b	16.1 ± 4.69 ^b	15.3 ± 5.01 ^b	0.119	0.593	0.146
21		15.3 ± 4.61 ^b	14.1 ± 3.91 ^b	15.4 ± 4.89 ^b	15.2 ± 3.90 ^b	0.435	0.527	0.348
P		0.039	0.04	0.037	0.041			
GGT (U/L)		0	32.9 ± 5.16 ^{Aa}	29.2 ± 4.39 ^{Aa}	41.6 ± 4.92 ^{Ba}	38.2 ± 5.25 ^{Ba}	0.047	0.004
	3	29.6 ± 5.58 ^{Aa}	31.1 ± 4.50 ^{Aa}	45.9 ± 3.94 ^{Ba}	40.6 ± 4.39 ^{Ba}	0.045	0.235	0.269
	6	44.4 ± 4.12 ^b	43.6 ± 3.71 ^b	44.3 ± 5.62 ^b	44.9 ± 4.38 ^b	0.091	0.978	0.101
	9	43.3 ± 4.39 ^b	44.4 ± 3.58 ^b	45.2 ± 4.14 ^b	42.4 ± 4.09 ^b	0.635	0.160	0.158
	12	39.6 ± 4.65 ^b	42.5 ± 4.28 ^b	41.4 ± 4.09 ^b	40.7 ± 5.83 ^b	0.806	0.140	0.056
	15	46.3 ± 6.01 ^b	45.4 ± 5.36 ^b	42.1 ± 4.61 ^b	42.9 ± 5.08 ^b	0.602	0.090	0.105
	18	45.9 ± 4.11 ^b	39.3 ± 4.43 ^b	45.9 ± 5.44 ^b	42.4 ± 4.15 ^b	0.479	0.146	0.195
	21	40.3 ± 5.34 ^b	39.6 ± 4.33 ^b	39.7 ± 5.81 ^b	39.8 ± 4.81 ^b	0.986	0.574	0.382
	P	0.036	0.048	0.014	0.024			
	Urea (mg/dL)	0	24.4 ± 5.42	22.7 ± 5.80	26.1 ± 4.47	24.1 ± 2.11	0.830	0.081
3		24.6 ± 3.50	26.5 ± 3.16	24.6 ± 3.07	24.3 ± 4.01	0.985	0.329	0.587
6		26.2 ± 5.87	26.9 ± 4.39	24.7 ± 4.23	25.5 ± 3.46	0.712	0.171	0.371
9		26.3 ± 3.70	24.7 ± 2.26	24.0 ± 5.96	26.8 ± 2.88	0.691	0.493	0.540
12		26.8 ± 5.16	25.0 ± 3.72	26.0 ± 3.24	25.9 ± 2.62	0.518	0.995	0.340
15		24.3 ± 5.94	25.9 ± 3.92	24.0 ± 5.51	25.5 ± 4.84	0.711	0.336	0.192
18		26.2 ± 5.72	24.1 ± 4.15	26.3 ± 4.90	24.7 ± 3.12	0.510	0.010	0.082
21		26.4 ± 4.44	25.0 ± 2.11	26.9 ± 2.76	25.0 ± 2.09	0.180	0.400	0.296
P		0.216	0.231	0.317	0.411			
Uric acid (mg/dL)		0	0.5 ± 0.01	0.6 ± 0.08	0.6 ± 0.02	0.5 ± 0.09	0.560	0.001
	3	0.5 ± 0.03	0.5 ± 0.06	0.6 ± 0.06	0.5 ± 0.04	0.682	0.609	0.108
	6	0.3 ± 0.01	0.3 ± 0.09	0.3 ± 0.04	0.4 ± 0.05	0.786	0.233	0.712
	9	0.2 ± 0.08	0.2 ± 0.07	0.2 ± 0.04	0.2 ± 0.02	0.809	0.285	0.631
	12	0.2 ± 0.08	0.3 ± 0.03	0.3 ± 0.02	0.3 ± 0.07	0.546	0.125	0.083
	15	0.4 ± 0.02	0.3 ± 0.06	0.2 ± 0.03	0.2 ± 0.02	0.649	0.098	0.835
	18	0.3 ± 0.01	0.4 ± 0.01	0.2 ± 0.02	0.3 ± 0.07	0.833	0.457	0.738
	21	0.4 ± 0.02	0.2 ± 0.05	0.3 ± 0.03	0.2 ± 0.05	0.703	0.647	0.904
	P	0.649	0.809	0.842	0.703			
	LDL (mg/dL)	0	8.80 ± 1.45 ^{Aa}	5.09 ± 2.39 ^{Aa}	12.7 ± 2.65 ^{Ba}	13.1 ± 2.61 ^{Ba}	0.018	0.018
3		20.2 ± 5.20 ^b	19.0 ± 3.49 ^b	21.7 ± 1.09 ^b	21.8 ± 1.64 ^b	0.301	0.026	0.057
6		22.8 ± 1.87 ^b	20.1 ± 1.71 ^b	21.0 ± 2.17 ^b	20.6 ± 4.02 ^b	0.518	0.059	0.130
9		22.7 ± 5.58 ^{Ab}	20.1 ± 4.61 ^{Ab}	16.7 ± 3.83 ^{Bb}	14.0 ± 4.02 ^{Bb}	0.027	0.174	0.944
12		28.7 ± 2.90 ^b	27.7 ± 1.75 ^b	28.2 ± 4.61 ^b	29.1 ± 5.04 ^b	0.915	0.232	0.253
15		21.3 ± 3.43 ^{Ab}	20.0 ± 1.38 ^{Ab}	33.3 ± 1.51 ^{Bb}	31.8 ± 1.90 ^{Bb}	0.034	0.002	0.507
18		18.7 ± 2.32 ^b	19.0 ± 3.15 ^b	21.4 ± 2.73 ^b	20.4 ± 1.42 ^b	0.082	0.884	0.014
21		22.3 ± 3.16 ^b	21.7 ± 1.34 ^b	24.3 ± 5.62 ^b	22.4 ± 2.84 ^b	0.071	0.378	0.019
P		0	0	0	0			
HDL (mg/dL)		0	10.6 ± 2.24 ^{Aa}	14.5 ± 3.90 ^{Aa}	20.7 ± 3.27 ^{Ba}	23.6 ± 2.56 ^{Ba}	0.024	<0.001
	3	32.5 ± 3.50 ^b	31.3 ± 4.78 ^b	32.8 ± 3.56 ^b	31.4 ± 2.71 ^b	0.315	0.108	0.188
	6	31.4 ± 4.15 ^b	28.1 ± 3.45 ^b	28.8 ± 4.56 ^b	29.6 ± 2.47 ^b	0.615	0.992	0.237
	9	34.4 ± 3.03 ^{Ab}	33.9 ± 4.63 ^{Ab}	28.9 ± 3.37 ^{Bb}	26.0 ± 4.67 ^{Bb}	0.031	0.850	0.737
	12	37.1 ± 4.52 ^b	39.3 ± 4.67 ^b	36.0 ± 2.25 ^b	32.6 ± 3.58 ^b	0.811	0.064	0.109
	15	33.1 ± 3.78 ^{Ab}	38.2 ± 2.45 ^{Ab}	41.5 ± 4.28 ^{Bb}	44.3 ± 3.35 ^{Bb}	0.048	0.063	0.121
	18	40.9 ± 4.09 ^b	41.6 ± 6.33 ^b	35.6 ± 4.92 ^b	36.8 ± 3.34 ^b	0.072	0.365	0.361
	21	36.4 ± 4.27 ^b	39.1 ± 5.75 ^b	41.4 ± 4.83 ^b	40.8 ± 5.36 ^b	0.081	0.103	0.084
	P	0	0	0	0			

Variables	Day	Treatments				P		
		Holstein		Simmental		Treat	Mineral	Breed
		Control	Mineral	Control	Mineral			
Cho (mg/dL)	0	20.0 ± 3.72 ^{Aa}	21.1 ± 3.54 ^{Aa}	40.6 ± 5.62 ^{Ba}	38.8 ± 4.07 ^{Ba}	0.016	0.434	0.056
	3	59.1 ± 6.08 ^b	57.1 ± 4.81 ^b	57.1 ± 5.79 ^b	58.4 ± 3.30 ^b	0.212	0.220	0.266
	6	60.1 ± 5.39 ^b	56.8 ± 3.08 ^b	55.3 ± 6.49 ^b	57.5 ± 4.07 ^b	0.727	0.709	0.641
	9	71.1 ± 6.94 ^{Ab}	70.7 ± 3.51 ^{Ab}	67.9 ± 4.15 ^{Bb}	64.3 ± 5.05 ^{Bb}	0.042	0.044	0.040
	12	74.6 ± 5.70 ^b	77.3 ± 3.61 ^b	72.0 ± 7.22 ^b	71.2 ± 2.71 ^b	0.903	0.101	0.153
	15	71.1 ± 8.61 ^{Ab}	76.0 ± 6.50 ^{Ab}	80.9 ± 8.25 ^{Bb}	89.7 ± 7.56 ^{Bb}	0.034	0.215	0.160
	18	72.6 ± 6.24 ^b	80.3 ± 7.98 ^b	71.4 ± 7.70 ^b	70.9 ± 8.09 ^b	0.061	0.082	0.088
	21	72.3 ± 5.98 ^b	75.7 ± 3.77 ^b	80.2 ± 6.30 ^b	79.6 ± 8.03 ^b	0.074	0.030	0.012
	P	0	0	0	0			
	Glu (mg/dL)	0	80.7 ± 7.30 ^a	85.7 ± 5.32 ^a	80.3 ± 1.90 ^a	75.9 ± 4.82 ^a	0.829	0.006
3		82.3 ± 3.20 ^a	79.3 ± 3.46 ^a	85.7 ± 4.62 ^a	75.5 ± 3.46 ^a	0.869	0.891	0.257
6		79.8 ± 5.14 ^a	80.7 ± 1.54 ^a	75.1 ± 7.81 ^a	78.6 ± 7.02 ^a	0.812	0.445	0.093
9		76.1 ± 6.55 ^a	77.3 ± 4.18 ^a	80.4 ± 2.54 ^a	79.0 ± 5.15 ^a	0.830	0.503	0.179
12		81.8 ± 6.11 ^a	82.6 ± 7.92 ^a	87.2 ± 4.52 ^a	83.4 ± 3.58 ^a	0.802	0.088	0.347
15		87.1 ± 3.11 ^a	89.9 ± 3.51 ^a	90.1 ± 7.44 ^a	83.3 ± 2.28 ^a	0.808	0.198	0.284
18		83.2 ± 3.37 ^a	81.1 ± 1.30 ^a	92.5 ± 5.08 ^a	90.5 ± 2.62 ^a	0.867	0.303	0.738
21		72.1 ± 7.78 ^b	68.1 ± 7.41 ^b	74.1 ± 5.65 ^b	71.1 ± 4.24 ^b	0.785	0.118	0.085
P		0	0	0	0			
Trig (mg/dL)		0	25.1 ± 0.34	28.5 ± 0.12	30.4 ± 0.24	35.1 ± 0.12	0.801	<0.001
	3	18.6 ± 0.30	19.6 ± 0.48	19.2 ± 0.52	18.2 ± 0.30	0.840	0.230	0.058
	6	16.3 ± 0.53	15.2 ± 0.64	16.3 ± 0.71	16.3 ± 0.53	0.765	0.117	0.182
	9	16.6 ± 0.81	17.0 ± 0.85	18.1 ± 0.86	16.3 ± 0.71	0.122	0.267	0.237
	12	24.3 ± 0.71	24.9 ± 0.67	20.7 ± 0.47	22.6 ± 0.81	0.814	0.512	0.089
	15	26.8 ± 0.52	21.3 ± 0.82	28.2 ± 0.78	21.8 ± 0.51	0.822	0.504	0.334
	18	21.4 ± 0.71	20.3 ± 0.88	25.3 ± 1.14	23.4 ± 0.14	0.765	0.924	0.442
	21	24.1 ± 0.76	21.2 ± 0.76	26.5 ± 0.78	24.1 ± 0.16	0.512	0.060	0.137
	P	0.502	0.306	0.067	0.126			
	TP (mg/dL)	0	5.55 ± 0.16	5.21 ± 0.45	6.79 ± 0.13	6.34 ± 0.02	0.739	0.034
3		5.44 ± 0.15	5.57 ± 0.18	6.41 ± 0.14	6.12 ± 0.17	0.555	0.160	0.026
6		5.07 ± 0.14	5.51 ± 0.13	6.60 ± 0.15	6.08 ± 0.15	0.815	0.148	0.130
9		5.76 ± 0.11	5.66 ± 0.14	6.74 ± 0.10	6.76 ± 0.11	0.716	0.792	0.092
12		5.03 ± 0.15	5.98 ± 0.19	6.56 ± 0.14	6.03 ± 0.15	0.123	0.051	0.141
15		7.06 ± 0.16	7.32 ± 0.18	6.58 ± 0.17	6.06 ± 0.16	0.432	0.156	0.144
18		7.38 ± 0.17	7.21 ± 0.06	7.03 ± 0.15	6.38 ± 0.17	0.235	0.959	0.973
21		7.56 ± 0.14	7.40 ± 0.15	6.80 ± 0.11	6.56 ± 0.14	0.487	0.206	0.025
P		0.076	0.084	0.075	0.082			

Note: ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyl transferase; LDL, low-density lipoprotein; HDL, high-density lipoprotein; Chol, total cholesterol; Glu, glucose; Trig, triglyceride; TP, total protein. Different superscripts, capital letters in rows for treatment differences, small letters in columns for time differences, indicate statistically significant differences (p<0.05).

Table 3. Serum cortisol values (µg/mL) (0-21st days) of Holstein and Simmental calves in response to organic minerals supplementation

Day	Treatments				P		
	Holstein		Simmental		Time	Mineral	Beed
	Control	Mineral	Control	Mineral			
0	1079 ± 55.1 ^a	984 ± 34.2 ^a	915 ± 47.2 ^a	951 ± 42.8 ^a	0.815	0.017	0.137
3	897 ± 25.7 ^a	815 ± 15.7 ^a	912 ± 22.7 ^a	811 ± 29.1 ^a	0.645	<0.001	0.609
6	672 ± 77.1 ^b	677 ± 34.3 ^b	672 ± 31.4 ^b	651 ± 12.8 ^b	0.712	0.223	0.062
9	371 ± 22.3 ^b	350 ± 20.1 ^b	353 ± 21.6 ^b	343 ± 20.3 ^b	0.115	0.006	0.016
12	305 ± 20.5 ^b	339 ± 18.5 ^b	333 ± 22.4 ^b	324 ± 23.6 ^b	0.367	0.080	0.409
15	350 ± 20.6 ^{Ab}	293 ± 20.5 ^{Bb}	353 ± 25.1 ^{Ab}	224 ± 11.8 ^{Bb}	0.021	<0.001	<0.001
18	286 ± 34.8 ^{Ac}	115 ± 15.7 ^{Bc}	212 ± 11.6 ^{Ac}	111 ± 18.2 ^{Bc}	0.014	<0.001	<0.001
21	197 ± 43.9 ^{Ac}	178 ± 24.8 ^{Bc}	118 ± 29.7 ^{Ac}	71.0 ± 18.2 ^{Bc}	0.011	<0.001	<0.001
P	0.032	0.014	0.023	0.016			

Note: Different superscripts, capital letters in rows for treatment differences, small letters in columns for time differences, indicate statistically significant differences (p<0.05).

Based on the results of our study, the mixture of organic zinc, chromium, and selenium added to colostrum and milk from birth to weaning in both Holstein and Simmental calves did not alter the

blood total protein, urea, or uric acid concentrations. Furthermore, urea nitrogen levels in the calves did not exceed 26.96 mg/dL throughout the study period. These results are similar to those previously reported

Table 4. Serum cortisol values of Holstein and Simmental calves ($\mu\text{g/mL}$) (28-63rd days) in response to organic minerals supplementation

Day	Treatments				P		
	Holstein		Simmental		Time	Mineral	Breed
	Control	Mineral	Control	Mineral			
28	345 \pm 21.5 ^a	351 \pm 27.2 ^a	387 \pm 23.6 ^a	357 \pm 21.2 ^a	0.815	0.171	0.003
35	312 \pm 20.1 ^a	346 \pm 25.4 ^a	348 \pm 22.5 ^a	349 \pm 25.6 ^a	0.645	0.025	0.010
42	550 \pm 20.6 ^b	447 \pm 20.5 ^b	567 \pm 23.1 ^b	597 \pm 25.1 ^b	0.712	0.128	<0.001
49	471 \pm 22.3 ^a	350 \pm 20.1 ^a	353 \pm 21.6 ^a	343 \pm 20.3 ^a	0.115	0.892	0.869
56	305 \pm 20.5 ^a	339 \pm 18.5 ^a	333 \pm 22.4 ^a	324 \pm 23.6 ^a	0.367	0.130	0.119
63	350 \pm 20.6 ^a	293 \pm 20.5 ^a	353 \pm 25.1 ^a	224 \pm 11.8 ^a	0.812	<0.001	<0.001
P	0.048	0.036	0.047	0.037			

Note: Different superscripts in columns for time differences indicate statistically significant differences ($p < 0.05$).

Table 5. Serum IgG concentrations of Holstein and Simmental calves (mg/dL) in response to organic minerals supplementation

Day	Treatments				P		
	Holstein		Simmental		Time	Breed	Mineral
	Control	Mineral	Control	Mineral			
0	2.40 \pm 0.42 ^a	2.55 \pm 0.33 ^a	2.25 \pm 0.24 ^a	2.35 \pm 0.37 ^a	0.814	0.049	0.142
14	1.3 \pm 0.44 ^b	1.2 \pm 0.12 ^b	1.5 \pm 0.37 ^b	1.3 \pm 0.29 ^b	0.812	0.005	0.006
21	0.8 \pm 0.02 ^c	0.6 \pm 0.07 ^c	0.9 \pm 0.06 ^c	0.7 \pm 0.02 ^c	0.547	0.074	0.003
P	0.023	0.018	0.032	0.025			

Note: Different superscripts in columns for time differences indicate statistically significant differences ($p < 0.05$).

for healthy calves (Pekcan *et al.*, 2023). Furthermore, according to the literature, organic zinc and methionine were given to newborn calves for 8 weeks, which did not affect the serum total protein level, consistent with the findings of our study (Ülger & Küçük, 2011).

Total cholesterol, triglyceride, HDL, and LDL concentrations in serum are important variables that provide information about metabolism in newborn calves. Additionally, the fact that serum lipid and lipoprotein levels were within normal limits in farm animals indicates that the animals were in a healthy and normal physiological state (Motta *et al.*, 2023; Żarczyńska *et al.*, 2021). Piccione *et al.* (2010) found that the serum total cholesterol levels of calves nearly doubled in the first 5 days after birth and increased to approximately four times the level seen at birth by 30 days of age. However, triglyceride levels did not change despite an increase during the first 30 days. Pekcan *et al.* (2023) reported that while the serum cholesterol level was 112.57 ± 11.88 mg/dL in healthy calves, this level decreased to 57.33 ± 4.61 mg/dL in animals with arthritis and diarrhea. In the present study, cholesterol, HDL, and LDL levels increased during the first 21 days after birth in both Simmental and Holstein calves, with no changes thereafter. Similarly, serum triglyceride levels did not change from birth until weaning, which is consistent with the findings of Piccione *et al.* (2010). The total cholesterol levels observed in the present study increased with time, in parallel with the levels specified for healthy calves close to the weaning period, and approached the blood cholesterol level of adult cattle. This outcome is consistent with the cholesterol levels documented for calves (Pekcan *et al.*, 2023) and for adult cattle (Alameen *et al.*, 2012) in previous studies. Likewise, the addition of organic zinc to calves' diet for 8 weeks from birth did not change the blood

cholesterol and triglyceride (Ülger & Küçük, 2011); the calves' blood cholesterol and triglyceride levels reported previously were similar to those observed in the current study. These findings are consistent with those of previous studies that demonstrated the beneficial effects of dietary modifications in managing lipid profiles in hyperlipidemic models (Iqbal *et al.*, 2024). Although concrete data on neonatal calves are limited, enhanced metabolic responses to organic minerals may be associated with changes in biochemical parameters, including lipids and liver enzyme stability, suggesting enhanced hepatic function. Additionally, an enhanced metabolic status with organic trace mineral sources has been linked with improved overall biochemical profiles, which could plausibly reflect triglyceride, LDL, HDL, and cholesterol dynamics in neonatal calves receiving such supplementation. Moreover, the interplay between inflammation and lipid metabolism is evident in various studies, highlighting the importance of considering both metabolic and inflammatory pathways when investigating lipid-lowering interventions (Wang *et al.*, 2022).

Studies have demonstrated the effectiveness of organic salts and hyperosmotic sodium bicarbonate in correcting metabolic acidosis in neonatal calves, providing insights into how metabolic balance and correction can be restored through targeted interventions, thereby aiding overall health improvements (Humayun *et al.*, 2022). Blood glucose levels are one of the most important variables of metabolic health and facilitate the determination of rumen development in calves and the level of absorption from the rumen wall, especially regarding volatile fatty acid production in the rumen. Calves are born monogastric, and their rumen develops over time as they transition into multistomached ruminants. After

this stage, blood glucose levels are determined by the digestion of nutrients in the rumen, the synthesis of essential fatty acids, and their utilization in gluconeogenesis in the liver. One of the minerals used in this study is organic chromium. Chromium positively affects glucose utilization primarily by enhancing the effect of insulin as a glucose tolerance factor (Khare *et al.*, 2023). Blood glucose levels do not change significantly within the first 30 days after birth (Piccione *et al.*, 2010). However, a different study argued that as calves are born functionally monogastric, their blood glucose levels tend to decrease (Wenker *et al.*, 2022). The findings obtained in the present study showed a decrease in blood glucose levels in calves, especially in the first 21 days after birth, which is in agreement with previous reports (Piccione *et al.*, 2010), regardless of whether they were Holstein or Simmental. Similarly, supplementation of calves with organic zinc did not affect their blood glucose levels (Ülger & Küçük, 2011).

Blood cortisol levels increase because of the organism's reaction to various internal and external factors that cause stress (Kim *et al.*, 2022). Several studies have reported that blood cortisol levels increase with stress and gradually decrease with the disappearance of stress (Kim *et al.*, 2011; Kovács *et al.*, 2021; Slayi & Jaja, 2025). For example, blood cortisol levels increase significantly in sheep because of stress during shearing (Kotianová *et al.*, 2025). In the present study, blood cortisol levels, which were very high at birth, decreased in the first 21 days in all groups, regardless of the breed. However, they increased on the days when weaning was initiated (after the 42nd day of the study) and decreased again after weaning. Breed-related differences in serum IgG levels between Holstein and Simmental calves likely reflect the inherent variation in colostrum quality and the efficiency with which immunoglobulins are absorbed at birth, as breed has been identified as a major factor influencing colostrum IgG concentrations and passive transfer to neonates. Genetic selection pressures and metabolic specialization also differ between these breeds, with Holsteins being intensely selected for high milk yield and Simmentals having a more dual-purpose background, potentially shaping early immune and metabolic trajectories and baseline IgG status in neonatal calves (Cavirani *et al.*, 2024). Breed differences in stress physiology may exhibit variations in the hypothalamic–pituitary–adrenal axis activity. Because cortisol interacts closely with immune modulation and inflammatory adaptation in early life, such differences in cortisol dynamics may contribute to the divergent IgG and cortisol responses observed in calves supplemented with organic minerals (Sgorlon *et al.*, 2015). The observed differences in serum cortisol and IgG levels have important implications for early calf growth. Higher serum IgG levels in newborn calves have been reported to be associated with better passive immunity and a higher average daily gain at an early age, indicating that calves with better immunity grow faster in early life (Elsohaby *et al.*, 2019). Conversely, higher cortisol levels are indicative of higher stress levels and can suppress immune function and appetite,

with potential adverse effects on average daily gain and overall growth (Hulbert & Moisés, 2016). Therefore, breed-specific differences in stress responses and immune status, as well as the potential benefits of organic mineral supplementation on physiological markers, may translate into meaningful variations in the growth of neonatal calves.

Almost all factors that contribute to immunity in newborn calves are acquired via the colostrum (Lopez & Heinrichs, 2022). A vital proportion (80%) of the antibodies contained in the colostrum is constituted by IgG (Johnson *et al.*, 2007). Therefore, IgG levels in calf blood in the first few weeks after birth provide essential information regarding passive immunization, which is the primary defense against infections in calves (Rocha *et al.*, 2012). Previous studies have shown that organic mineral supplementation has a positive effect on immunity levels in farm animals (Zhang *et al.*, 2021). Adding organic or inorganic chromium to the diets of calves increases blood IgG levels (Kumar *et al.*, 2023). The findings of the current study indicate that the level of maternal antibodies acquired at birth decreases over time, regardless of breed. Additionally, organic mineral supplementation did not have a significant positive or negative effect on this decline. However, its effects may become apparent when active immunity develops, subsequently affecting the immune system. This study emphasizes the importance of passive immunization in calves from the beginning to the end of oral administration.

CONCLUSION

Serum ALT, AST, GGT, HDL, LDL, and total cholesterol levels in Simmental calves, especially in the first week of life, were higher than those in Holstein calves. However, this difference decreased over the following weeks. Serum IgG levels were not affected; however, organic mineral supplementation decreased serum cortisol levels in both Simmental and Holstein calves throughout the study. As an increase in serum cortisol levels is the primary and most significant response to stress, our results indicate that organic minerals may help protect newborn calves against oxidative stress. However, studies covering the active immunity period induced through vaccination are required to determine the precise influence of supplementing calves with organic minerals in terms of both immunity and antistress effects.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

No generative AI tool and AI-assisted technologies were used for the preparation of this manuscript.

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