

Article

Investigation of Effects of Low Ruminal pH Values on Serum Concentrations of Macrominerals, Trace Elements, and Vitamins and Oxidative Status of Dairy Cows

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Simple Summary

This study investigated how low ruminal pH affects the blood concentrations of macrominerals, trace elements, and vitamins and the oxidative status of dairy cows in early lactation. Ruminal pH often drops below 5.5 during the day. Blood samples and ruminal fluid were collected from 53 Holstein cows at three points in early lactation (days 30, 90, and 150). The obtained results show that low pH did not significantly affect most minerals, vitamins, or oxidative stress markers. However, cows with low pH had higher levels of copper, iron, and cobalt in their blood—especially by day 150. In brief, low ruminal pH changes some trace minerals but does not impact overall vitamin levels or oxidative status.

Abstract

Due to the feeding system (high-concentrate diet) during the early lactation stage, ruminal pH in dairy cows follows a diurnal pattern and can remain below the critical level of 5.5 for extended periods of the day. This study aimed to evaluate the effect of low ruminal pH on blood concentrations of certain macrominerals, trace minerals, and fat-soluble vitamins and on the oxidative status of dairy cows during the first half of lactation. Fifty-three randomly selected lactating Holstein cows were used; blood and ruminal fluid samples were collected from all cows on days 30, 90 and 150 of lactation. Blood samples were obtained via coccygeal venipuncture, while the ruminal fluid was obtained by rumenocentesis and the pH was measured immediately after collection. Using a threshold pH of 5.5, samples were classified as normal (pH > 5.5) or low pH (pH ≤ 5.5). Serum concentrations of Ca, Mg, K, Cr, Mn, Zn, Se, and vitamins A, D3, E, and K were not significantly affected by ruminal pH, either by days in milk or by their interaction ($p > 0.05$). Plasma malondialdehyde and reduced glutathione followed the same trend ($p > 0.05$). Copper concentration was



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significantly higher ($p < 0.05$), and Fe concentration tended to be higher in cows with low pH compared to those with normal pH ($p = 0.052$). On day 150 of lactation, Cu, Fe, and Co concentrations were significantly higher in low-pH cows compared to normal-pH cows ($p < 0.05$). Low ruminal pH is associated with significant changes in serum concentrations of copper, iron, and cobalt but has no significant effect on the oxidative status of the animals or on the serum concentrations of the macro elements and fat-soluble vitamins studied.

Keywords: trace elements; macro elements; serum vitamins; oxidative stress; ruminal fluid pH; dairy cattle

1. Introduction

Rumen plays a central role in the digestion and nutrient metabolism of ruminants, providing the primary site for microbial fermentation and nutrient absorption [1]. One of the most critical factors influencing ruminal function is pH, which directly affects microbial population dynamics, fermentation patterns, and the bioavailability of essential nutrients [2]. Under physiological conditions, rumen pH is tightly regulated; however, dietary changes, especially the inclusion of high-concentrate feeds, can lead to fluctuations that predispose dairy cows to subacute or acute ruminal acidosis. These pH alterations not only compromise feed efficiency and milk production but may also have systemic consequences extending beyond the gastrointestinal tract [3]. A reduction in ruminal pH has been demonstrated to influence the composition of rumen bacterial populations, patterns of fermentation, and concentrations of rumen fatty acids [4]. Such alterations may also impact the bioavailability of specific nutrients, including macrominerals, fat-soluble vitamins, and trace elements, which may subsequently influence their concentrations in the blood serum [5,6]. Furthermore, there is evidence in the literature indicating that low ruminal pH induces an inflammatory response and leads to oxidative stress due to an increase in reactive oxygen species [7,8]. This may necessitate an augmented requirement for nutrients to facilitate antioxidant processes, including vitamin E, Se, Zn, Mn, and Cu [9]. Previous studies have primarily focused on the impact of ruminal pH on fermentation end-products and animal performance, while fewer have examined its effects on systemic biochemical parameters. In particular, the influence of varying rumen pH levels on the serum concentrations of macrominerals (e.g., calcium, phosphorus, magnesium), trace elements (e.g., copper, zinc, selenium), fat-soluble vitamins (e.g., vitamins A and E), and oxidative stress markers remains insufficiently elucidated [10–12]. These parameters are critical not only for maintaining metabolic homeostasis but also for immune function, reproductive performance, and antioxidant defense mechanisms [13,14].

Trace elements are indispensable for the maintenance of health, immune function, growth, production, and reproduction [15]. Copper (Cu) and manganese (Mn) function as cofactors in a number of enzymatic reactions and are involved in lipid metabolism [16,17]. Chromium (Cr) plays a significant role in glucose metabolism as a glucose tolerance factor [18] and in lipid metabolism by reducing cortisol and non-esterified fatty acids (NEFAs) [19]. Iron (Fe) is an essential trace element for cows, as it is responsible for blood production, oxidative and energy metabolism, immune regulation, and the inflammatory response [20]. Selenium (Se) and zinc (Zn) contain multiple antioxidant and immune components and are involved in numerous physiological processes through metalloenzymes [17,21]. Furthermore, Zn has been demonstrated to markedly enhance milk yield [22]. Cobalt (Co) is essential for maintaining the cobalamin-producing ruminal microbiota and ruminal metabolism [23]. Given that trace minerals such as Se, Cu, and Zn are integral

components of antioxidant enzymes (e.g., glutathione peroxidase, superoxide dismutase), alterations in their metabolism due to low ruminal pH may impair oxidative defense mechanisms. Thus, the interaction between rumen pH, mineral availability, and oxidative stress represents a tightly interlinked regulatory axis with implications for systemic health and productivity in dairy cows [24].

In addition to trace elements, vitamins are responsible for several biological functions, including antioxidant activity, growth, milk production, fertility, and immunity [25]. Vitamin A plays a role in pathogen resistance and epithelial tissue defense [26]. Furthermore, it has been demonstrated that vitamin A supplementation enhances milk production and immune function in cows [27]. Vitamin D has been demonstrated to stimulate the innate immune system and maintain calcium and phosphorus homeostasis [28]. Similarly, in addition to its critical effects on the immune system and fertilization, vitamin E is known to increase macrophage and neutrophil function, reduce somatic cell count (SCC), and improve mammary health [29,30]. Although vitamin K is widely recognized for its role in blood coagulation, recent research has also demonstrated the beneficial effects of vitamin K supplementation on lactation performance, ruminal fermentation, and the gut microbiome [31,32].

In light of the pivotal role of macrominerals, vitamins, and trace elements in the health and productivity of dairy cows, and given the prevalence of low ruminal pH, we hypothesized that exposure to low ruminal pH alters the serum concentrations of macrominerals, trace elements, and fat-soluble vitamins and increases oxidative stress in cows. This study aimed to examine the impact of low ruminal pH on the blood concentration of these parameters and on the oxidative status of dairy cows.

2. Materials and Methods

2.1. Animals and Experimental Design

The study was conducted on a Greek commercial dairy farm with high prevalence (50%, farm#11, [33]) of subacute ruminal acidosis (SARA) and is considered a part of a previous study [4]. The lactating animals on the farm were housed collectively in a two-row free stall barn. The animals were provided with a total mixed ration (TMR) at an ad libitum level, with the composition of the TMR adjusted throughout the study in accordance with the herd's milk production level. The TMR consisted of the following components: corn silage (45% of dry matter (DM)), alfalfa hay (11% of DM), wheat straw (4–9% of DM), corn grain (12–15% of DM), wheat bran (6% of DM), soybean meal (12–14% of DM), Ca-soap fat supplement (1.5% of DM), and a premix of vitamins and minerals (3.5% of DM). This premix contained 20.79% Ca, 3.37% P, 7.50% Mg, 6% Na, 600,000 IU/kg vitamin A, 120,000 IU/kg vitamin D3, 3 g/kg vitamin E, 1.44 g/kg ZnO, 2.85 g/kg Zn chelate, 1.86 g/kg MnO, 1.98 g/kg Mn chelate, 0.675 g/kg Cu chelate, 0.18 g/kg I, 0.04 g/kg Co and 0.02 g Se. The TMR was formulated in accordance with the net energy and metabolizable protein recommendations set forth by the National Research Council [34]. The feed was supplemented with sodium bicarbonate (0.75% DM); however, no other buffer was included. The bunk space for lactating cows was 60 cm, and the stocking density for the lactating group ranged from 90% to 125% throughout the study period.

A total of 53 clinically healthy lactating Holstein cows were randomly selected for inclusion in the study. The median parity was 1.49 (SD: 0.69), with 19 multiparous and 34 primiparous cows. The study period spanned a duration of 120 days, commencing at 30 days in milk (DIM) and concluding at 150 DIM. On days 30, 90, and 150 of lactation, blood samples and a sample of ruminal fluid were collected after a comprehensive clinical examination of the animals. A clinical examination was conducted whenever the farmer observed the presence of a clinical disease or a reduction in milk production.

Rumen fluid was collected via rumenocentesis, and the pH was immediately measured as previously described [4]. Rumenocentesis was consistently performed between 12:00 and 14:00, in order to be within the suggested timeframe of 5–8 h after morning feeding [35]. The pH status of cows at each rumenocentesis was categorized as either low pH (pH values ≤ 5.5) or normal pH (pH values > 5.5) using the aforementioned cut-off value of 5.5 [36].

2.2. Blood Collection and Laboratory Analysis

Blood was collected via coccygeal venipuncture into 10 mL evacuated polyethylene tubes containing K3EDTA (BD Vacutainer® BD, Franklin Lakes, NJ, USA) and not containing anticoagulant (BD Vacutainer® BD, Franklin Lakes, NJ, USA) following the morning milking and prior to feeding. The samples were transported to the laboratory on ice, whereupon the serum/plasma was separated by low-speed centrifugation ($1600\times g$ for 20 min) approximately two to three hours after collection. Subsequently, the serum/plasma was transferred to Eppendorf tubes and stored at -80°C . Serum samples were utilized for the quantification of Ca, P, Mg, K, Zn, Cu, Fe, Mn, Co, Se, Cr, and vitamins A, D3, E, and K. Additionally, the concentrations of MDA and GSH were determined in plasma.

2.3. Macro and Trace Element Analysis

The macro and trace element analysis of the serum samples was carried out by using an inductively coupled plasma–optical emission spectrophotometer (ICP-OES; Thermo iCAP 6000 series; Thermo Fisher Co., Cambridge, UK) at the Cerrahpasa Faculty of Medicine, Istanbul University-Cerrahpasa. In order to carry out the trace element analysis, serum samples were diluted (1:10) with distilled water in a sterile disposable polyethylene tube using an automatic pipette. ICP-OES device parameters for the determination of elements are presented in Table 1. For quality assurance of the ICP-OES analysis, we used proper test solutions containing 2000 ppm (mg/L) for each tested element obtained from Chem-Lab NV. Standard solutions of all elements were prepared by taking appropriate amounts of standards containing 1000 ppm (mg/L) for each tested element obtained from Chem-Lab NV in deionized water. Using standard and blank solutions as reference material, 3-point calibration was performed. Reproducible and linear calibration curves were obtained for analysis, and the correlation coefficient of the calibration curve was found for each of the elements measured. The calibration graph was obtained from the ICP-OES device using blank and standard solutions, and element-level analyses of the prepared samples were carried out.

Table 1. ICP-OES device parameters for determination of trace and toxic elements.

Parameters	Assigned Value
Plasma gas flow rate	15 L/min
Argon carrier flow rate	0.5 L/min
Sample flow rate	1.51 L/min
The speed of the peristaltic pump	100 rpm
RF power	1150 W

In the study, appropriate wavelengths of Cr (267.716 nm), Cu (324.754 nm), Fe (259.940 nm), Mg (285.213 nm), Mn (257.610 nm), Se (196.090 nm), Zn (206.200 nm), Co (228.616 nm), Ca (317.933 nm), P (213.618 nm), and K (766.490 nm) were used for analysis with the ICP-OES device. Each measurement was repeated three times and averaged for analysis.

2.4. Vitamin (A, D, E, and K) Analysis

Alpha-tocopherol, retinol, phylloquinone, and cholecalciferol stock solutions were prepared at 500 $\mu\text{g}/\text{mL}$. To prepare a standard solution, the stock solutions were diluted

suitably with methanol. To determine proper calibration, linear regression analysis of the peak area was used to standardize the solution concentrations.

To minimize sample degradation due to exposure to UV light, the samples, which were covered with plastic sleeves, were thawed at ambient temperature under fluorescent light. Retinol, cholecalciferol, α -tocopherol, and phyloquinone in serum were extracted as follows: 100 μ L serum was deproteinized by adding 100 μ L ethanol and antioxidants such as 0.025% BHT to the extraction solvent. The samples were mixed via vortexing for 1 min [37]. The samples were extracted twice with 600 μ L n-hexane. After mixing the samples via vortexing, they were centrifuged at 8000 rev/min for 10 min. A total of 500 μ L of hexane layer was extracted, and it was evaporated under a nitrogen stream of 37 °C to dryness. The residue was dissolved in 50 μ L tetrahydrofuran and was added to 150 μ L methanol. After vortexing the samples for 1 min, 100 μ L samples were autosampled using amber glass vials.

The chromatographic system consisted of HP Agilent 1100 with a G-1328 Diode Array Detector (DAD) and G1329 ALS autosampler (−8 °C). Agilent Technologies HP software version B.04.03 was used to process the data. A 5 μ m GI Science C18 reversed-phase column (250 $\times$ 4.6 mm ID) was used for separation. Then, the mobile phase of a methanol–tetrahydrofuran mixture (80:20, *v/v*) was modified [38]. The pump was adjusted to achieve a flow rate of 1.5 mL/min. Chromatographic analysis was performed at 40 °C using isocratic elution. The chromatogram was monitored with DAD array detection at 290, 325, 265, and 248 nm for the simultaneous measurement of α -tocopherol, retinol, cholecalciferol, and phyloquinone, respectively.

2.5. Malondialdehyde (MDA) Determination

Plasma was extracted from the blood for oxidative stress assessments. The resulting samples, comprising malondialdehyde (MDA) from lipid peroxidation reacting with thio-barbituric acid (TBA), were measured according to the method of Draper and Hadley [39]. Each plasma sample (0.25 mL) was mixed with 1 mL of TCA (75%), 1.5 mL of TBA (30%), and 0.2 mL of HCL (5 M), then incubated at 95 °C for 15 min. After reaching room temperature, the samples were centrifuged at 10,000 rpm for 10 min, and the supernatants were measured at a wavelength of 532 nm using a spectrophotometer (Shimadzu UV-1800). Absorbance values for MDA were obtained from the spectrophotometer device. MDA results were given in μ mol/L.

2.6. Glutathione (GSH) Determination

For antioxidant measurements, plasma was obtained from the blood. GSH levels were measured using Beutler's method [31], which involves coloration with Ellman's reagent (DTNB) [5,5'-Dithiobis (2-nitrobenzoic acid)]. First, 0.3 mL of the precipitation solution was added to a 0.2 mL sample and centrifuged at 4000 rpm for 5 min. Then, 0.5 mL of secondary phosphate solution and 0.1 mL of Ellman's reagent were added to the 0.2 mL supernatant obtained after centrifugation, and the mixture was incubated for 5 min at room temperature. The GSH levels were then analyzed using a spectrophotometer (Shimadzu UV-1800, Shimadzu, Tokyo, Japan) at a wavelength of 412 nm. Absorbance values for GSH were recorded, and the results were expressed in μ mol/L.

2.7. Data Analysis

The statistical software Jeffreys' Amazing Statistics Program JASP (v 0.18.3) was used for data analysis. A two-way ANOVA was performed to assess the effect of pH status, DIM, and their interaction on the biochemical parameters evaluated. Homogeneity of variances was assessed using Levene's test and the normality of the data was assessed by evaluating Q-Q plots of the residuals. Post hoc analysis was performed using the Tukey test. A significance level of $p \leq 0.05$ was used for all comparisons.

3. Results

The frequency of low-pH cows on DIM 30 was 45.3% (24/53); on DIM 90, it was 54.71% (29/53); and on DIM 150, it was 66% (35/53).

The mean calcium concentrations remained unaffected by the pH status and DIM ($p > 0.05$; Table 2). However, although no significant difference was detected between the pH status of cows on the sampling days, the mean values on DIM 30 exhibited a tendency toward higher levels in cows with a low pH compared to those with normal pH ($p = 0.058$). The mean P concentration was found to be only significantly affected by DIM ($p < 0.05$), exhibiting a significant increase from DIM 30 to DIM 90 (Table 2). No significant effect was observed for pH status, and no significant difference was detected among the pH status of cows on the sampling days. The mean Mg and K concentrations were not significantly affected by either pH status or DIM ($p > 0.05$; Table 2). However, on DIM 150, the mean Mg values of low-pH cows were significantly higher than those of normal-pH cows ($p < 0.05$).

Table 2. Mean $\pm$ SE serum concentrations of calcium (Ca), inorganic phosphorus (P), magnesium (Mg), and potassium (K) in cows with low (pH ≤ 5.5) and normal (pH > 5.5) ruminal pH on the three sampling days—days 30, 90, and 150 of lactation (DIM)—and the mean $\pm$ SE values according to ruminal pH status for each DIM.

Parameter		DIM			pH Status Mean
		30	90	150	
Ca ($\mu\text{g/mL}$)	Low pH	90.03 $\pm$ 2.54	85.77 $\pm$ 2.45	90.41 $\pm$ 2.45	88.73 $\pm$ 1.44
	Normal pH	83.27 $\pm$ 2.45	88.27 $\pm$ 2.59	89.67 $\pm$ 2.90	87.07 $\pm$ 1.53
	DIM mean	86.65 $\pm$ 1.77	87.02 $\pm$ 1.80	90.04 $\pm$ 1.90	
P ($\mu\text{g/mL}$)	Low pH	117.47 $\pm$ 7.34	144.51 $\pm$ 7.20	162.56 $\pm$ 7.07	141.51 $\pm$ 4.16
	Normal pH	115.83 $\pm$ 7.07	145.14 $\pm$ 7.48	152.06 $\pm$ 8.37	137.68 $\pm$ 4.42
	DIM mean	116.65 $\pm$ 5.10 ^A	144.82 $\pm$ 5.19 ^B	157.31 $\pm$ 5.48 ^B	
Mg ($\mu\text{g/mL}$)	Low pH	20.78 $\pm$ 0.67	20.70 $\pm$ 0.66	21.35 $\pm$ 0.65	20.95 $\pm$ 0.38
	Normal pH	20.72 $\pm$ 0.65	21.34 $\pm$ 0.68	19.23 $\pm$ 0.77	20.43 $\pm$ 0.40
	DIM mean	20.75 $\pm$ 0.47 ^A	21.02 $\pm$ 0.48 ^A	20.29 $\pm$ 0.502 ^A	
K ($\mu\text{g/mL}$)	Low pH	108.04 $\pm$ 6.69	120.07 $\pm$ 6.69	103.85 $\pm$ 6.43	110.65 $\pm$ 3.81
	Normal pH	94.58 $\pm$ 6.55	111.90 $\pm$ 6.55	102.25 $\pm$ 7.52	102.91 $\pm$ 4.00
	DIM mean	101.31 $\pm$ 4.68	115.98 $\pm$ 4.68 ^A	103.05 $\pm$ 4.94	

Different uppercase letters within rows indicate statistically significant differences among DIM points ($p < 0.05$).

The mean copper concentration was found to be significantly higher in cows with low pH levels compared to those with normal pH levels (mean $\pm$ SE: 10.55 $\pm$ 0.32 and 9.45 $\pm$ 0.32 $\mu\text{mol/L}$ for low- and normal-pH cows, respectively; $p < 0.05$). No significant difference was observed between sampling days (mean $\pm$ SE: 9.61 $\pm$ 0.32, 10.08 $\pm$ 0.32, and 10.24 $\pm$ 0.32 $\mu\text{mol/L}$ for DIM 30, 90, and 150, respectively; $p > 0.05$). As illustrated in Figure 1, serum copper concentration exhibited no statistically significant difference between animals with different pH status on DIM 30 and 90 ($p > 0.05$). However, on DIM 150, it was observed to be significantly higher ($p < 0.05$) in low-pH cows compared to normal-pH cows (Figure 1). The mean iron concentration was not significantly affected by either pH status or DIM (mean $\pm$ SE: 23.63 $\pm$ 1.61, 27.57 $\pm$ 1.61, and 24.70 $\pm$ 1.61 $\mu\text{mol/L}$ for DIM 30, 90, and 150, respectively; $p > 0.05$). However, there was a tendency for the low-pH cows to exhibit higher levels than the normal-pH cows (mean $\pm$ SE: 27.03 $\pm$ 1.25 and 23.45 $\pm$ 1.25 $\mu\text{mol/L}$ for low- and normal-pH cows, respectively; $p = 0.052$). The mean Fe values were found to be significantly higher in the low-pH animals compared to those with normal pH on DIM 150 (Figure 2; $p < 0.05$). No other differences were observed between the groups on the other sampling days ($p > 0.05$). Cobalt concentrations remained

practically stable among days in milk (DIM) (mean $\pm$ SE: 80.05 ± 14.02 , 56.29 ± 14.15 , and 75.31 ± 14.79 nmol/L for DIM 30, 90, and 150, respectively; $p > 0.05$) and were not significantly affected by pH status (mean $\pm$ SE: 83.13 ± 11.38 and 57.97 ± 12.01 nmol/L for low- and normal-pH cows, respectively; $p < 0.05$). However, on day 150 of the experiment, the mean values were found to be significantly higher in the animals with low pH levels compared to those with normal pH levels on DIM 150 (Figure 3; $p < 0.05$). No significant difference was observed among the pH status groups on the other DIM (Figure 3; $p > 0.05$). As indicated in Table 3, the mean serum concentrations of Cr, Se, Mn, and Zn were not significantly influenced by pH status or DIM. Furthermore, no significant differences were observed between groups on any sampling day ($p > 0.05$).

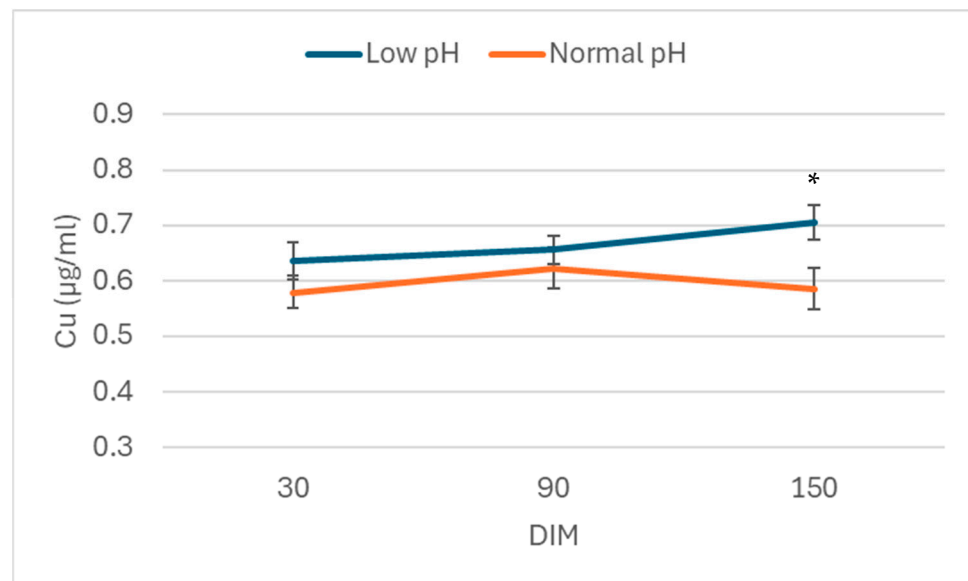


Figure 1. Mean $\pm$ SE serum concentrations of copper (Cu) in cows with low ($\text{pH} \leq 5.5$) and normal ($\text{pH} > 5.5$) ruminal pH on the three sampling days—days 30, 90, and 150 of lactation (DIM). * significant difference ($p < 0.05$).

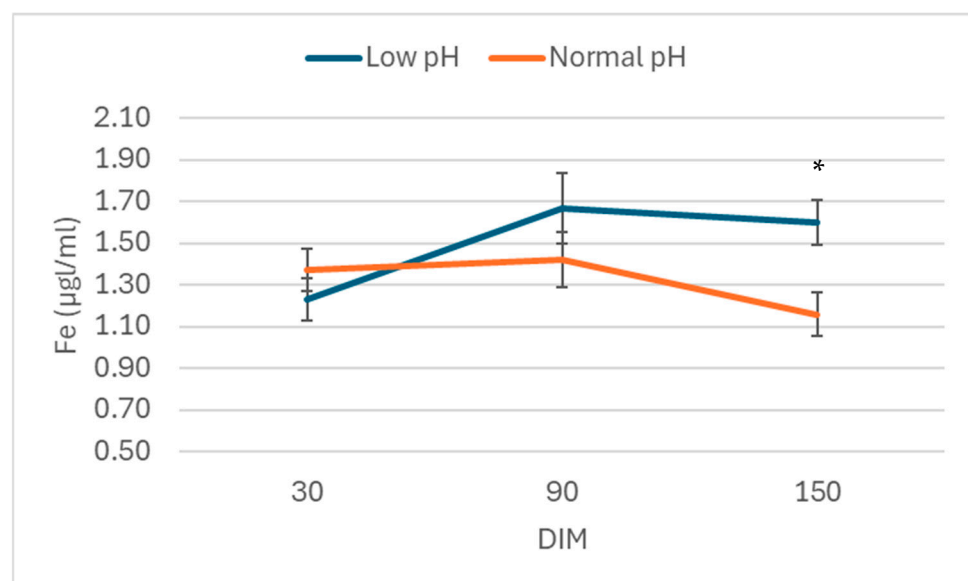


Figure 2. Mean $\pm$ standard error serum concentrations of serum iron (Fe) in cows with low ($\text{pH} \leq 5.5$) and normal ($\text{pH} > 5.5$) ruminal pH on the three sampling days—days 30, 90, and 150 of lactation (DIM). * Significant difference ($p < 0.05$).

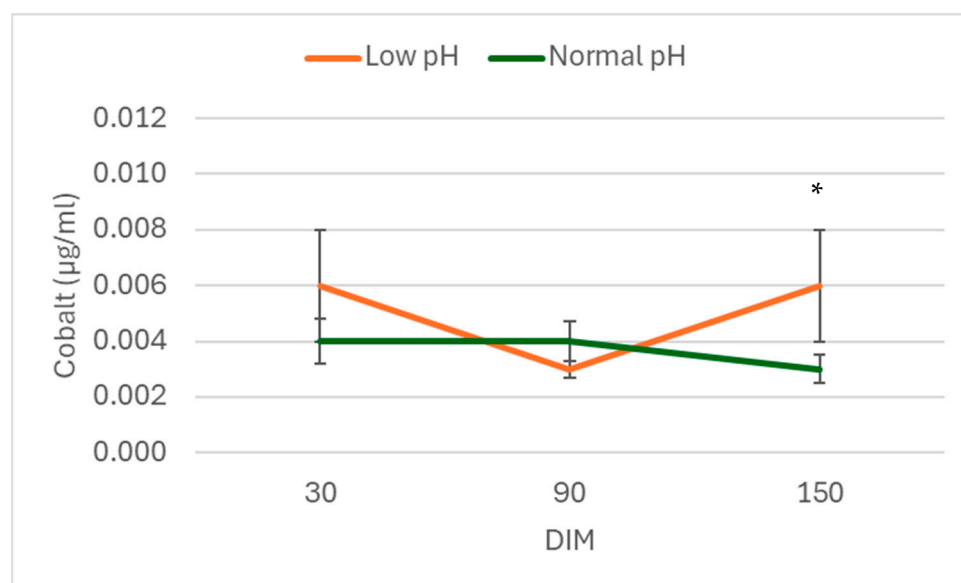


Figure 3. Mean $\pm$ SE serum concentrations of serum cobalt (Co) in cows with low ($\text{pH} \leq 5.5$) and normal ($\text{pH} > 5.5$) ruminal pH on the three sampling days, days 30, 90, and 150 of lactation (DIM). * Significant difference ($p < 0.05$).

Table 3. Mean $\pm$ standard error serum concentrations of chromium (Cr), manganese (Mn), selenium (Se), and zinc (Zn) in cows with low ($\text{pH} \leq 5.5$) and normal ($\text{pH} > 5.5$) ruminal pH on the three sampling days—days 30, 90, and 150 of lactation (DIM)—and mean $\pm$ SE values according to ruminal pH status for each DIM.

Parameter		DIM			pH Status Mean
		30	90	150	
Cr ($\mu\text{g/mL}$)	Low pH	0.032 $\pm$ 0.010	0.032 $\pm$ 0.010	0.039 $\pm$ 0.009	0.034 $\pm$ 0.006
	Normal pH	0.036 $\pm$ 0.009	0.027 $\pm$ 0.010	0.020 $\pm$ 0.011	0.028 $\pm$ 0.006
	DIM mean	0.034 $\pm$ 0.007	0.029 $\pm$ 0.007	0.029 $\pm$ 0.007	
Mn ($\mu\text{g/mL}$)	Low pH	0.004 $\pm$ 0.0006	0.003 $\pm$ 0.0006	0.005 $\pm$ 0.0006	0.004 $\pm$ 0.0004
	Normal pH	0.003 $\pm$ 0.0006	0.004 $\pm$ 0.0006	0.004 $\pm$ 0.0007	0.004 $\pm$ 0.0004
	DIM mean	0.004 $\pm$ 0.0004	0.004 $\pm$ 0.0004	0.004 $\pm$ 0.0005	
Se ($\mu\text{g/mL}$)	Low pH	0.457 $\pm$ 0.030	0.469 $\pm$ 0.029	0.483 $\pm$ 0.029	0.470 $\pm$ 0.017
	Normal pH	0.399 $\pm$ 0.029	0.496 $\pm$ 0.030	0.459 $\pm$ 0.034	0.451 $\pm$ 0.018
	DIM mean	0.428 $\pm$ 0.021	0.483 $\pm$ 0.021	0.471 $\pm$ 0.022	
Zn ($\mu\text{g/mL}$)	Low pH	0.507 $\pm$ 0.027	0.500 $\pm$ 0.026	0.572 $\pm$ 0.026	0.526 $\pm$ 0.015
	Normal pH	0.464 $\pm$ 0.026	0.519 $\pm$ 0.027	0.500 $\pm$ 0.030	0.494 $\pm$ 0.016
	DIM mean	0.485 $\pm$ 0.019	0.510 $\pm$ 0.019	0.536 $\pm$ 0.020	

No significant difference was detected either between ruminal pH groups or among DIM points ($p > 0.05$).

The mean concentrations of the fat-soluble vitamins evaluated, as well as those of MDA and GSH, remained practically stable throughout the study period and were not significantly affected by the animals' ruminal pH status ($p > 0.05$; Table 4). Moreover, no notable discrepancies were discerned between the various pH status groups on any given sampling day ($p > 0.05$).

Table 4. Mean $\pm$ standard error serum/plasma concentrations of vitamins A, D, E, and K, malondialdehyde (MDA), and glutathione (GSH) in cows with low (pH $\leq$ 5.5) and normal (pH $>$ 5.5) ruminal pH on the three sampling days—days 30, 90, and 150 of lactation (DIM)—and mean $\pm$ SE values according to ruminal pH status for each DIM.

Parameter		DIM			pH Status Mean
		30	90	150	
Vit. A ($\mu\text{g/mL}$)	Low pH	1.109 $\pm$ 0.141	1.042 $\pm$ 0.164	1.126 $\pm$ 0.128	1.092 $\pm$ 0.084
	Normal pH	1.208 $\pm$ 0.148	1.318 $\pm$ 0.141	1.092 $\pm$ 0.164	1.206 $\pm$ 0.082
	DIM mean	1.158 $\pm$ 0.102	1.180 $\pm$ 0.108	1.109 $\pm$ 0.104	
Vit. D ($\mu\text{g/mL}$)	Low pH	0.183 $\pm$ 0.025	0.178 $\pm$ 0.028	0.169 $\pm$ 0.022	0.177 $\pm$ 0.014
	Normal pH	0.160 $\pm$ 0.025	0.209 $\pm$ 0.024	0.165 $\pm$ 0.028	0.178 $\pm$ 0.015
	DIM mean	0.171 $\pm$ 0.018	0.193 $\pm$ 0.018	0.167 $\pm$ 0.018	
Vit. E ($\mu\text{g/mL}$)	Low pH	1.020 $\pm$ 0.121	1.195 $\pm$ 0.140	1.290 $\pm$ 0.114	1.168 $\pm$ 0.072
	Normal pH	0.943 $\pm$ 0.129	0.900 $\pm$ 0.124	1.096 $\pm$ 0.140	0.980 $\pm$ 0.076
	DIM mean	0.982 $\pm$ 0.089	1.048 $\pm$ 0.093	1.193 $\pm$ 0.090	
Vit. K ($\mu\text{g/mL}$)	Low pH	0.797 $\pm$ 0.110	0.715 $\pm$ 0.124	0.731 $\pm$ 0.099	0.748 $\pm$ 0.064
	Normal pH	0.752 $\pm$ 0.112	0.856 $\pm$ 0.107	0.699 $\pm$ 0.124	0.769 $\pm$ 0.066
	DIM mean	0.774 $\pm$ 0.078	0.785 $\pm$ 0.082	0.715 $\pm$ 0.079	
MDA ($\mu\text{mol/L}$)	Low pH	17.485 $\pm$ 1.155	15.526 $\pm$ 1.138	16.657 $\pm$ 1.155	16.556 $\pm$ 0.664
	Normal pH	16.022 $\pm$ 1.414	14.500 $\pm$ 1.106	16.868 $\pm$ 1.254	15.797 $\pm$ 0.730
	DIM mean	16.753 $\pm$ 0.913	15.013 $\pm$ 0.793	16.763 $\pm$ 0.852	
GSH ($\mu\text{mol/L}$)	Low pH	23.691 $\pm$ 2.962	23.018 $\pm$ 2.918	27.367 $\pm$ 2.962	24.692 $\pm$ 1.702
	Normal pH	26.880 $\pm$ 3.628	21.562 $\pm$ 2.836	26.474 $\pm$ 3.216	24.972 $\pm$ 1.872
	DIM mean	25.285 $\pm$ 2.342	22.290 $\pm$ 2.035	26.921 $\pm$ 2.186	

No significant difference was detected either between ruminal pH groups or among DIM points ($p > 0.05$).

4. Discussion

The objective of this study was to evaluate the impact of low ruminal pH on the blood concentration of macrominerals, trace minerals, and fat-soluble vitamins, as well as on the oxidative status of dairy cows during the initial 150 days following calving, encompassing the first half of the lactation period. The cows were classified as having either low or normal pH at each sampling point based on their ruminal pH. This is the reason why the number of animals in each category differs at each sampling point.

According to the TMR formulations, the cows' diet contained a high proportion of concentrates, and based on the amount of starch and effective NDF, the predicted ruminal pH was 6.3 [40]. The high frequency of low-pH cows in this study may have been induced by feeding changes throughout the study, diet presentation (e.g., small particles in the forage sources used), inadequate bunk space (60 cm and not the appropriate 75 cm per highly lactating cow), and occasionally increased stocking density, instead of the diet composition per se.

Rumen pH is a critical determinant of the microbial ecology and biochemical dynamics within the rumen, with downstream effects on mineral solubility, absorption, and vitamin synthesis. For instance, magnesium and calcium solubility has been shown to decrease at lower pH values due to alterations in ionized forms and precipitation dynamics, thereby reducing their ultrafilterable fractions in the rumen fluid [41]. A sustained reduction in ruminal pH alters the balance between cellulolytic and amylolytic bacteria, reducing the population of fiber-degrading species and favoring acid-tolerant, lactate-producing microbes [10,42]. These microbial shifts not only impair fiber digestion but may also suppress the synthesis of certain B vitamins, particularly cobalamin (vitamin B12), which requires cobalt as a precursor and is synthesized by rumen microbes under neutral pH

conditions [23,43]. Moreover, acidic ruminal conditions reduce the availability of sulfide, thereby disrupting the formation of thiomolybdate, a compound that modulates copper absorption by binding to molybdenum [6]. The altered thiomolybdate formation may thus increase apparent copper availability, as observed in the current study. Additionally, low ruminal pH increases epithelial permeability and facilitates the translocation of endotoxins into circulation, potentially inducing hepatic synthesis of acute-phase proteins such as ceruloplasmin and haptoglobin, which in turn affect trace mineral distribution, particularly copper and iron [44–46]. Collectively, these mechanisms suggest that ruminal acidosis has the potential to disturb the homeostasis of multiple micronutrients by altering both microbial activity and host physiology.

The results of the present study indicate that pH status has a negligible impact on the serum concentration of the macro elements in question. With regard to calcium, the results of previous studies are inconclusive. In accordance with our findings, ref. [47] observed no alteration in Ca concentration in SARA-induced animals. However, other studies have reported a significant reduction in serum Ca in cattle with spontaneous long-lasting SARA [48] and in SARA-induced animals [49]. In the aforementioned studies, a reduction in serum calcium was associated with endotoxemia and the detoxification of lipopolysaccharides that had been translocated into the bloodstream from the ruminal fluid. It is possible that, in the present study, either the low pH status of the animals was not long lasting or the production of lipopolysaccharides was not sufficiently elevated to impact serum calcium concentrations. In contrast to our observations, serum P was found to be significantly lower in cattle with spontaneous long-lasting SARA [48], and this was attributed to concurrent hypocalcemia. The significant increase in serum phosphorus concentrations between days 30 and 90 is probably associated with the increase in dry matter and phosphorus dietary intake since there is a linear relationship between phosphorous intake and its serum concentration [50].

The concentration of serum Cu was found to be significantly influenced by the ruminal pH status of the animals. Although plasma Cu concentration is considered an unreliable indicator of Cu status, this parameter may be useful in situations where animals experience markedly elevated or decreased hepatic Cu concentrations [51]. The present study demonstrated that cows with low pH exhibited significantly elevated Cu concentrations compared to those with normal pH. This effect may be associated with alterations in the ruminal environment that occur during periods of low pH, which in turn affect the Cu, molybdenum, and sulfur complex by altering the production of thiomolybdate. It is well documented that a reduction in ruminal pH results in a decrease in the availability of bisulfide, which is combined with molybdenum to form thiomolybdate [52]. It is plausible that the ruminal production of thiomolybdate is diminished in cows with low pH, which may consequently result in augmented apparent Cu availability and absorption and serum Cu concentration. Increased copper availability, determined by increased liver Cu concentration, has been observed in cattle fed potentially acidogenic high-starch diets, but this was not reflected in the plasma Cu concentration [53].

An additional potential explanation is that elevated serum copper levels in cows with low pH are linked to enhanced ceruloplasmin activity. Ceruloplasmin is a mild to moderate acute-phase protein that increases in response to inflammation [54,55] and contains more than 95% of the copper present in plasma [56]. It is well documented that feeding cows a high-concentrate diet induces an inflammatory response as a result of ruminal acidosis [8,57] and is associated with an increase in acute-phase proteins such as haptoglobin and serum amyloid-A [57]. An increase in ceruloplasmin was observed in dairy cows fed high-starch diets, yet the plasma Cu concentration remained unaltered [53]. The activity of ceruloplasmin and the concentration of copper both increase immediately

following calving, subsequently declining gradually during the early and middle stages of lactation [58]. It seems plausible that, in addition to the anticipated increase in ceruloplasmin activity during the early lactation period, cows with low pH had even higher levels of ceruloplasmin and, consequently, serum Cu than cows with normal pH. This may also account for the consistently elevated serum Cu concentrations observed in low-pH cows throughout the experimental period, as well as the significantly higher concentrations observed after 150 DIM, when ceruloplasmin activity is expected to be normalized in normal-pH cows.

In addition to copper, ceruloplasmin, as a ferroxidase, plays a role in iron metabolism, specifically in the mobilization of iron from tissue stores. An increase in ceruloplasmin has been observed to be associated with a proportional increase in serum iron concentration [59]. This hypothesis may also explain the alterations observed in serum iron concentrations in this study.

The decline in serum cobalt concentrations in cows with normal pH levels was gradual, which is consistent with previous observations in dairy cattle [60]. This decline was attributed to the removal of cobalt from milk during the lactation period. In cows with low pH, the serum cobalt concentration was elevated on DIM 150, exhibiting a significant difference compared to normal-pH cows. The reason for this increase is currently unknown. The lower milk production of low-pH cows would be a plausible explanation. However, this hypothesis cannot be supported since, as demonstrated in our previous study, which included the same animals, milk yield remained unaffected by ruminal pH status [61]. The absorption of cobalt in the lumen is closely associated with the absorption of other divalent cations, including copper, iron, and zinc, as they all utilize the divalent cation transporter, which is influenced by vitamin D [62,63]. Given that Cu and Fe were also significantly higher in low-pH cows on 150 DIM, an increase in the active transportation of these elements in low-pH animals could be a reason for this. However, this cannot be confirmed since the vitamin D concentrations were not significantly different among low- and normal-pH cows, and serum Zn levels were also unaffected. An additional potential explanation is that the alterations in the ruminal environment resulting from low pH do not support the accumulation of Co for vitamin B12 production by flora. This may result in an increased amount available for absorption, although this hypothesis lacks evidence and warrants further investigation.

Previous research has indicated that the long-term feeding of high-concentrate diets to dairy cows can lead to an increase in oxidative stress [8,64–66]. Furthermore, this is associated with increased plasma concentrations of MDA [8,64,65] and decreased plasma concentrations of GSH [8]. Consistent with the findings of Tsuchiya et al. [7], ruminal pH status did not affect plasma MDA and GSH concentrations in the present study. This may be attributed to the fact that the animals in the present study were classified according to pH status based on a single measurement on the sampling day. In addition, oxidative biomarkers are known to exhibit diurnal or circadian patterns and may be influenced by transient physiological stimuli, including feeding, stress, or metabolic stage [67,68]. Although all samples were collected consistently during a certain time window to minimize this variability, supporting valid group comparisons, the results may not fully reflect potential diurnal fluctuations in oxidative status. The comparable oxidative status between low- and normal-pH animals may also partially explain the similar concentrations of vitamin E detected in these animals. The absence of significant differences in serum concentrations of vitamins A and K indicates that low pH status, as defined here, does not negatively affect these vitamins.

Several limitations should be acknowledged when interpreting the results of the present study. Firstly, the concentrations of minerals and fat-soluble vitamins were mea-

sured only in serum samples. Direct quantification of these nutrients in the total mixed ration (TMR) and ruminal fluid was not performed. Consequently, potential relationships between dietary intake, ruminal content, and systemic availability could not be fully established. While the TMR formulation was standardized according to NRC [34] guidelines, variations in individual feed intake and nutrient bioavailability may have contributed to inter-animal variability in serum concentrations. Secondly, ruminal pH was assessed at discrete time points using rumenocentesis, which reflects only a snapshot of rumen acidity rather than the complete diurnal fluctuation. Although the categorization into “Low pH” and “Normal pH” groups provided useful comparisons, continuous pH monitoring could have yielded more comprehensive insights into the ruminal environment. Lastly, the study was conducted on a single commercial dairy farm with a relatively small sample size, which may limit the generalizability of the findings to broader populations and management systems. Further multicenter, longitudinal studies incorporating dietary, microbial, and immunological parameters are warranted to validate and expand upon these findings.

5. Conclusions

In the context of the present study, it was demonstrated that low ruminal pH values have no significant impact on the oxidative status of animals or on the serum concentrations of macro elements and fat-soluble vitamins. However, they are associated with significant alterations in the serum concentrations of copper, iron, and cobalt. Further in-field investigation is required to elucidate the mechanisms by which low ruminal pH alters the concentrations of these trace elements. These findings emphasize the importance of monitoring ruminal pH and trace element status—particularly copper, iron, and cobalt—in dairy cows, as suboptimal ruminal pH may alter the bioavailability or metabolism of these essential micronutrients. This suggests a need to adjust feeding strategies and supplementation protocols accordingly, especially in herds at risk of subacute ruminal acidosis, to maintain optimal health and productivity.

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References

1. Liu, K.; Zhang, Y.; Yu, Z.; Xu, Q.; Zheng, N.; Zhao, S.; Huang, G.; Wang, J. Ruminal Microbiota–Host Interaction and Its Effect on Nutrient Metabolism. *Anim. Nutr.* **2021**, *7*, 49–55. [[CrossRef](#)]
2. Faniyi, T.O.; Owolabi, A.; Soyelu, O.T. Rumen pH and Microbial Shift: Implications for Ruminant Nutrition—a Review. *Niger. J. Anim. Prod.* **2024**, *51*, 103–120. [[CrossRef](#)]

3. Elmhadi, M.E.; Ali, D.K.; Khogali, M.K.; Wang, H. Subacute Ruminal Acidosis in Dairy Herds: Microbiological and Nutritional Causes, Consequences, and Prevention Strategies. *Anim. Nutr.* **2022**, *10*, 148–155. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Kitkas, G.C.; Valergakis, G.E.; Kritsepi-Konstantinou, M.; Gelasakis, A.I.; Katsoulos, P.D.; Kalaitzakis, E.; Panousis, N.K. Association between Ruminal pH and Rumen Fatty Acids Concentrations of Holstein Cows during the First Half of Lactation. *Ruminants* **2022**, *2*, 382–389. [\[CrossRef\]](#)
5. Spears, J.W. Trace Mineral Bioavailability in Ruminants. *J. Nutr.* **2003**, *133*, 1506S–1509S. [\[CrossRef\]](#)
6. Underwood, E.J.; Suttle, N.F. *The Mineral Nutrition of Livestock*, 3rd ed.; CABI Publishing: Wallingford, UK, 1999; ISBN 978-0-85199-128-3.
7. Tsuchiya, Y.; Ozai, R.; Sugino, T.; Kawashima, K.; Kushibiki, S.; Kim, Y.-H.; Sato, S. Changes in Peripheral Blood Oxidative Stress Markers and Hepatic Gene Expression Related to Oxidative Stress in Holstein Cows with and without Subacute Ruminal Acidosis during the Periparturient Period. *J. Vet. Med. Sci.* **2020**, *82*, 1529–1536. [\[CrossRef\]](#)
8. Ma, N.; Abaker, J.A.; Wei, G.; Chen, H.; Shen, X.; Chang, G. A High-Concentrate Diet Induces an Inflammatory Response and Oxidative Stress and Depresses Milk Fat Synthesis in the Mammary Gland of Dairy Cows. *J. Dairy Sci.* **2022**, *105*, 5493–5505. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Koekkoek, W.A.C.; van Zanten, A.R. Antioxidant Vitamins and Trace Elements in Critical Illness. *Nutr. Clin. Pract.* **2016**, *31*, 457–474. [\[CrossRef\]](#)
10. Dijkstra, J.; Ellis, J.L.; Kebreab, E.; Strathe, A.B.; López, S.; France, J.; Bannink, A. Ruminal pH Regulation and Nutritional Consequences of Low pH. *Anim. Feed Sci. Technol.* **2012**, *172*, 22–33. [\[CrossRef\]](#)
11. Vargas, J.E.; López-Ferreras, L.; Andrés, S.; Mateos, I.; Horst, E.H.; López, S. Differential Diet and pH Effects on Ruminal Microbiota, Fermentation Pattern and Fatty Acid Hydrogenation in RUSITEC Continuous Cultures. *Fermentation* **2023**, *9*, 320. [\[CrossRef\]](#)
12. Xu, Y.; Ding, Z. Physiological, Biochemical and Histopathological Effects of Fermentative Acidosis in Ruminant Production: A Minimal Review. *Span. J. Agric. Res.* **2011**, *9*, 414–422. [\[CrossRef\]](#)
13. Chen, Y.-H.; Chen, Y.-M.; Tu, P.-A.; Lee, K.-H.; Chen, J.-Y.; Hsu, J.-T. Effect of Supplementing Vitamin E, Selenium, Copper, Zinc, and Manganese during the Transition Period on Dairy Cow Reproductive Performance and Immune Function. *Vet. Sci.* **2023**, *10*, 225. [\[CrossRef\]](#) [\[PubMed\]](#)
14. El-Sayed, A.; Ebissy, E.; Mohamed, R.; Ateya, A. Effects of Antioxidant Vitamins (A, D, E) and Trace Elements (Cu, Mn, Se, Zn) Administration on Gene Expression, Metabolic, Antioxidants and Immunological Profiles during Transition Period in Dromedary Camels. *BMC Vet. Res.* **2024**, *20*, 101. [\[CrossRef\]](#)
15. Yatoo, M.I.; Saxena, A.; Deepa, P.M.; Habeab, B.P.; Devi, S.; Jatav, R.S.; Dimri, U. Role of Trace Elements in Animals: A Review. *Vet. World* **2013**, *6*, 963–967. [\[CrossRef\]](#)
16. Engle, T.E. Copper and Lipid Metabolism in Beef Cattle: A Review. *J. Anim. Sci.* **2011**, *89*, 591–596. [\[CrossRef\]](#)
17. Gressley, T.F. Zinc, copper, manganese, and selenium in dairy cattle rations. In Proceedings of the 7th annual mid-Atlantic nutrition conference, Maryland, MD, USA, 26–27 March 2009; College Park: University of Maryland: Maryland, MD, USA.
18. Kretsinger, R.H.; Uversky, V.N.; Permyakov, E.A. *Encyclopedia of Metalloproteins*; Springer: New York, NY, USA, 2013; ISBN 978-1-4614-1532-9.
19. Malik, M.I.; Jonker, A.; Raboisson, D.; Song, B.; Rashid, M.A.; Sun, X. Effects of Dietary Chromium Supplementation on Blood Biochemical Parameters in Dairy Cows: A Multilevel Meta-Analytical Approach. *J. Dairy Sci.* **2024**, *107*, 301–316. [\[CrossRef\]](#)
20. Wysocka, D.; Snarska, A.; Sobiech, P.H. Iron in Cattle Health. *J. Elem.* **2020**, *25*, 1175–1185. [\[CrossRef\]](#)
21. Wang, R.L.; Liang, J.G.; Lu, L.; Zhang, L.Y.; Li, S.F.; Luo, X.G. Effect of Zinc Source on Performance, Zinc Status, Immune Response, and Rumen Fermentation of Lactating Cows. *Biol. Trace Elem. Res.* **2013**, *152*, 16–24. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Xu, N.N.; Yang, D.T.; Miao, C.; Valencak, T.G.; Liu, J.X.; Ren, D.X. Organic Zinc Supplementation in Early-Lactation Dairy Cows and Its Effects on Zinc Content and Distribution in Milk and Cheese. *JDS Commun.* **2021**, *2*, 110–113. [\[CrossRef\]](#) [\[PubMed\]](#)
23. González-Montaña, J.-R.; Escalera-Valente, F.; Alonso, A.J.; Lomillos, J.M.; Robles, R.; Alonso, M.E. Relationship between Vitamin B12 and Cobalt Metabolism in Domestic Ruminant: An Update. *Animals* **2020**, *10*, 1855. [\[CrossRef\]](#)
24. Hilal, E.Y.; Elkhairy, M.A.E.; Osman, A.O.A. The Role of Zinc, Manganese and Copper in Rumen Metabolism and Immune Function: A Review Article. *Open J. Anim. Sci.* **2016**, *6*, 304–324. [\[CrossRef\]](#)
25. Khan, M.Z.; Huang, B.; Kou, X.; Chen, Y.; Liang, H.; Ullah, Q.; Khan, I.M.; Khan, A.; Chai, W.; Wang, C. Enhancing Bovine Immune, Antioxidant and Anti-Inflammatory Responses with Vitamins, Rumen-Protected Amino Acids, and Trace Minerals to Prevent Periparturient Mastitis. *Front. Immunol.* **2024**, *14*, 1290044. [\[CrossRef\]](#)
26. Gürbüz, M.; Aktaş, Ş. Understanding the role of vitamin A and its precursors in the immune system. Available online: <https://www.em-consulte.com/article/1526806/article/understanding-the-role-of-vitamin-a-and-its-precu> (accessed on 28 June 2025).
27. Jin, L.; Yan, S.; Shi, B.; Bao, H.; Gong, J.; Guo, X.; Li, J. Effects of Vitamin A on the Milk Performance, Antioxidant Functions and Immune Functions of Dairy Cows. *Anim. Feed Sci. Technol.* **2014**, *192*, 15–23. [\[CrossRef\]](#)

28. Eder, K.; Grundmann, S.M. Vitamin D in Dairy Cows: Metabolism, Status and Functions in the Immune System. *Arch. Anim. Nutr.* **2022**, *76*, 1–33. [[CrossRef](#)] [[PubMed](#)]
29. Kuhn, M.J.; Sordillo, L.M. Vitamin E Analogs Limit In Vitro Oxidant Damage to Bovine Mammary Endothelial Cells. *J. Dairy Sci.* **2021**, *104*, 7154–7167. [[CrossRef](#)]
30. Xiao, J.; Khan, M.Z.; Ma, Y.; Alugongo, G.M.; Ma, J.; Chen, T.; Khan, A.; Cao, Z. The Antioxidant Properties of Selenium and Vitamin E; Their Role in Periparturient Dairy Cattle Health Regulation. *Antioxidants* **2021**, *10*, 1555. [[CrossRef](#)]
31. Bai, H.; Arai, H.; Ikuta, K.; Ishikawa, S.; Ohtani, Y.; Iwashita, K.; Okada, N.; Shirakawa, H.; Komai, M.; Terada, F.; et al. Effects of Dietary Vitamin K3 Supplementation on Vitamin K1 and K2 (Menaquinone) Dynamics in Dairy Cows. *Anim. Sci. J.* **2022**, *93*, e13680. [[CrossRef](#)] [[PubMed](#)]
32. Kuroiwa, T.; Ohtani, Y.; Obara, Y.; Terada, F.; Watanabe, K.; Shirakawa, H.; Komai, M.; Satoh, H.; Sato, S.; Ichijo, T. Effect of Vitamin K3 Supplementation on Immunoglobulin G Concentration in Colostrum of Periparturient Holstein Dairy Cows. *Anim. Sci. J.* **2022**, *93*, e13706. [[CrossRef](#)]
33. Kitkas, G.C.; Valergakis, G.E.; Karatzias, H.; Panousis, N. Subacute Ruminant Acidosis: Prevalence and Risk Factors in Greek Dairy Herds. *Ir. J. Vet. Res.* **2013**, *14*, 183–189. [[CrossRef](#)]
34. National Research Council, Committee on Animal Nutrition, and Subcommittee on Dairy Cattle Nutrition. *Nutrient Requirements of Dairy Cattle: Eighth Revised Edition*; National Academies Press: Washington, DC, USA, 2021; ISBN 978-0-309-67777-6.
35. Garrett, E.F.; Pereira, M.N.; Nordlund, K.V.; Armentano, L.E.; Goodger, W.J.; Oetzel, G.R. Diagnostic Methods for the Detection of Subacute Ruminant Acidosis in Dairy Cows. *J. Dairy Sci.* **1999**, *82*, 1170–1178. [[CrossRef](#)]
36. Oetzel, G.R. Monitoring and Testing Dairy Herds for Metabolic Disease. *Vet. Clin. N. Am. Food Anim. Pract.* **2004**, *20*, 651–674. [[CrossRef](#)]
37. Su, Q.; Rowley, K.G.; Balazs, N.D.H. Carotenoids: Separation Methods Applicable to Biological Samples. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2002**, *781*, 393–418. [[CrossRef](#)]
38. Siluk, D.; Oliveira, R.V.; Esther-Rodriguez-Rosas, M.; Ling, S.; Bos, A.; Ferrucci, L.; Wainer, I.W. A Validated Liquid Chromatography Method for the Simultaneous Determination of Vitamins A and E in Human Plasma. *J. Pharm. Biomed. Anal.* **2007**, *44*, 1001–1007. [[CrossRef](#)]
39. Draper, H.H.; Hadley, M. Malondialdehyde Determination as Index of Lipid Peroxidation. *Methods Enzymol.* **1990**, *186*, 421–431. [[CrossRef](#)] [[PubMed](#)]
40. Khorrami, B.; Khiaosa-ard, R.; Zebeli, Q. Models to Predict the Risk of Subacute Ruminant Acidosis in Dairy Cows Based on Dietary and Cow Factors: A Meta-Analysis. *J. Dairy Sci.* **2021**, *104*, 7761–7780. [[CrossRef](#)] [[PubMed](#)]
41. Martens, H.; Schweigel, M. Pathophysiology of Grass Tetany and Other Hypomagnesemias. Implications for Clinical Management. *Vet. Clin. N. Am. Food Anim. Pract.* **2000**, *16*, 339–368. [[CrossRef](#)]
42. Plaizier, J.C.; Krause, D.O.; Gozho, G.N.; McBride, B.W. Subacute Ruminant Acidosis in Dairy Cows: The Physiological Causes, Incidence and Consequences. *Vet. J.* **2008**, *176*, 21–31. [[CrossRef](#)] [[PubMed](#)]
43. Santschi, D.E.; Berthiaume, R.; Matte, J.J.; Mustafa, A.F.; Girard, C.L. Fate of Supplementary B-Vitamins in the Gastrointestinal Tract of Dairy Cows. *J. Dairy Sci.* **2005**, *88*, 2043–2054. [[CrossRef](#)]
44. Steele, M.A.; AlZahal, O.; Hook, S.E.; Croom, J.; McBride, B.W. Ruminant Acidosis and the Rapid Onset of Ruminant Parakeratosis in a Mature Dairy Cow: A Case Report. *Acta. Vet. Scand.* **2009**, *51*, 39. [[CrossRef](#)]
45. Gulhar, R.; Ashraf, M.A.; Jialal, I. Physiology, Acute Phase Reactants. In *StatPearls*; StatPearls Publishing: Treasure Island, FL, USA, 2025.
46. Spiers, J.G.; Tan, L.S.; Anderson, S.T.; Hill, A.F.; Lavidis, N.A.; Chen, H.-J.C. Hepatic Homeostasis of Metal Ions Following Acute Repeated Stress Exposure in Rats. *Antioxidants* **2021**, *11*, 85. [[CrossRef](#)]
47. Li, S.; Gozho, G.N.; Gakhar, N.; Khafipour, E.; Krause, D.O.; Plaizier, J.C. Evaluation of Diagnostic Measures for Subacute Ruminant Acidosis in Dairy Cows. *Can. J. Anim. Sci.* **2012**, *92*, 353–364. [[CrossRef](#)]
48. Morar, D.; Văduva, C.; Morar, A.; Imre, M.; Tulcan, C.; Imre, K. Paraclinical Changes Occurring in Dairy Cows with Spontaneous Subacute Ruminant Acidosis under Field Conditions. *Animals* **2022**, *12*, 2466. [[CrossRef](#)]
49. Danscher, A.M.; Li, S.; Andersen, P.H.; Khafipour, E.; Kristensen, N.B.; Plaizier, J.C. Indicators of Induced Subacute Ruminant Acidosis (SARA) in Danish Holstein Cows. *Acta. Vet. Scand.* **2015**, *57*, 39. [[CrossRef](#)] [[PubMed](#)]
50. Knowlton, K.F.; Herbein, J.H. Phosphorus Partitioning During Early Lactation in Dairy Cows Fed Diets Varying in Phosphorus Content1. *J. Dairy Sci.* **2002**, *85*, 1227–1236. [[CrossRef](#)]
51. Dias, R.S.; López, S.; Montanholi, Y.R.; Smith, B.; Haas, L.S.; Miller, S.P.; France, J. A Meta-Analysis of the Effects of Dietary Copper, Molybdenum, and Sulfur on Plasma and Liver Copper, Weight Gain, and Feed Conversion in Growing-Finishing Cattle1. *J. Anim. Sci.* **2013**, *91*, 5714–5723. [[CrossRef](#)]
52. Clarke, N.J.; Laurie, S.H. The Copper-Molybdenum Antagonism in Ruminants. I. The Formation of Thiomolybdates in Animal Rumen. *J. Inorg. Biochem.* **1980**, *12*, 37–43. [[CrossRef](#)] [[PubMed](#)]

53. McCaughern, J.H.; Mackenzie, A.M.; Sinclair, L.A. Dietary Starch Concentration Alters Reticular pH, Hepatic Copper Concentration, and Performance in Lactating Holstein-Friesian Dairy Cows Receiving Added Dietary Sulfur and Molybdenum. *J. Dairy Sci.* **2020**, *103*, 9024–9036. [[CrossRef](#)] [[PubMed](#)]
54. Sheldon, I.M.; Noakes, D.E.; Rycroft, A.; Dobson, H. Acute Phase Protein Responses to Uterine Bacterial Contamination in Cattle after Calving. *Vet. Rec.* **2001**, *148*, 172–175. [[CrossRef](#)]
55. Szczubial, M.; Dąbrowski, R.; Kankofer, M.; Bochniarz, M.; Albera, E. Concentration of Serum Amyloid A and Activity of Ceruloplasmin in Milk from Cows with Clinical and Subclinical Mastitis. *Bull. Vet. Inst. Pulawy* **2008**, *52*, 391–395.
56. Hellman, N.E.; Gitlin, J.D. Ceruloplasmin metabolism and function. *Annu. Rev. Nutr.* **2002**, *22*, 439–458. [[CrossRef](#)]
57. Zhao, C.; Liu, G.; Li, X.; Guan, Y.; Wang, Y.; Yuan, X.; Sun, G.; Wang, Z.; Li, X. Inflammatory Mechanism of Rumenitis in Dairy Cows with Subacute Ruminal Acidosis. *BMC Vet. Res.* **2018**, *14*, 135. [[CrossRef](#)]
58. Hussein, H.A.; Staufenbiel, R. Variations in Copper Concentration and Ceruloplasmin Activity of Dairy Cows in Relation to Lactation Stages with Regard to Ceruloplasmin to Copper Ratios. *Biol. Trace Elem. Res.* **2012**, *146*, 47–52. [[CrossRef](#)]
59. Gaware, V.; Kotade, K.; Dhamak, K.; Somawanshi, S. Ceruloplasmin its role and significance: A review. *Int. J. Biomed. Res.* **2010**, *5*, 10–7439. [[CrossRef](#)]
60. Kincaid, R.L.; Lefebvre, L.E.; Cronrath, J.D.; Socha, M.T.; Johnson, A.B. Effect of Dietary Cobalt Supplementation on Cobalt Metabolism and Performance of Dairy Cattle. *J. Dairy Sci.* **2003**, *86*, 1405–1414. [[CrossRef](#)]
61. Kitkas, G.C.; Valergakis, G.E.; Kritsepi-Konstantinou, M.; Gelasakis, A.I.; Arsenos, G.; Kalaitzakis, E.; Panousis, N. Effects of Ruminal pH and Subacute Ruminal Acidosis on Milk Yield and Composition of Holstein Cows in Different Stages of Lactation. *J. Hell. Vet. Med. Soc.* **2019**, *70*, 1551–1560. [[CrossRef](#)]
62. Schwalfenberg, G.K.; Genuis, S.J. Vitamin D, Essential Minerals, and Toxic Elements: Exploring Interactions between Nutrients and Toxicants in Clinical Medicine. *Sci. World J.* **2015**, *2015*, 318595. [[CrossRef](#)] [[PubMed](#)]
63. Brewer, K.; Maylin, G.; Fenger, C.; Tobin, T. Cobalt Use and Regulation in Horseracing: A Review. *Comp. Exerc. Physiol.* **2016**, *12*, 1–10. [[CrossRef](#)]
64. Guo, Y.; Xu, X.; Zou, Y.; Yang, Z.; Li, S.; Cao, Z. Changes in Feed Intake, Nutrient Digestion, Plasma Metabolites, and Oxidative Stress Parameters in Dairy Cows with Subacute Ruminal Acidosis and Its Regulation with Pelleted Beet Pulp. *J. Anim. Sci. Biotechnol.* **2013**, *4*, 31. [[CrossRef](#)]
65. Abaker, J.A.; Xu, T.L.; Jin, D.; Chang, G.J.; Zhang, K.; Shen, X.Z. Lipopolysaccharide Derived from the Digestive Tract Provokes Oxidative Stress in the Liver of Dairy Cows Fed a High-Grain Diet. *J. Dairy Sci.* **2017**, *100*, 666–678. [[CrossRef](#)]
66. Zhang, H.; Xue, Y.; Xie, W.; Wang, Y.; Ma, N.; Chang, G.; Shen, X. Subacute Ruminal Acidosis Downregulates FOXA2, Changes Oxidative Status, and Induces Autophagy in the Livers of Dairy Cows Fed a High-Concentrate Diet. *J. Dairy Sci.* **2023**, *106*, 2007–2018. [[CrossRef](#)] [[PubMed](#)]
67. Colakoglu, H.E.; Yazlik, M.O.; Kaya, U.; Colakoglu, E.C.; Kurt, S.; Oz, B.; Bayramoglu, R.; Vural, M.R.; Kuplulu, S. MDA and GSH-Px Activity in Transition Dairy Cows Under Seasonal Variations and Their Relationship with Reproductive Performance. *J. Vet. Res.* **2017**, *61*, 497–502. [[CrossRef](#)] [[PubMed](#)]
68. Younis, M.; El-Ashker, M.; El-Diasty, M.; Youssef, M.; El-khodery, S. Oxidative Stress in Transition Dairy Cattle: Current Knowledge and the Potential Impact of Supplementing Organic Trace Elements. *Asian J. Res. Anim. Vet. Sci.* **2021**, *7*, 1–21. [[CrossRef](#)]

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