Tropical Animal Health and Production (2022) 54:54
https://doi.org/10.1007/s11250-022-03061-6

REGULAR ARTICLES

# Dietary vitamin C in pre-parturient dairy cows and their calves: blood metabolites, copper, zinc, iron, and vitamin C concentrations, and calves growth performance

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Received: 15 June 2021 / Accepted: 4 January 2022


## Abstract

The aim of this study was to evaluate the effects of dietary vitamin C supplementation on blood parameters of pre-parturient (PP) dairy cows and growth performance and immune system of their newborn calves. Forty PP cows (at approximately 21 days before calving and an average weight 791 ± 50 kg) were allocated into two experimental treatments: (1) basal diet without vitamin C supplementation (CO) and (2) basal diet with 20 g of vitamin C supplementation from 21 days before calving to parturition (VC). After parturition, the experiment continued by grouping the calves into four dietary treatments with 8 calves in each treatment. The experimental treatments were (1) control calves with no vitamin C supplementation and from cows that received no vitamin C supplement (CON), (2) calves supplemented with 600 mg of vitamin C per day and from cows that received no vitamin C supplement (CVC), (3) calves supplemented with no vitamin C and from cows that received 20 g of vitamin C per day (MVC), and (4) calves supplemented with 600 mg of vitamin C per day and from cows that received 20 g of vitamin C per day (CMVC). Serum concentrations of glucose, HDL and LDL, cholesterol, triglycerides, total protein, and albumin of cows were not affected by vitamin C supplementation during pre-parturient period. However, cows that received VC diet had lower ($P < 0.05$) malondialdehyde (MDA) and aspartate aminotransferase (AST) concentrations, higher total antioxidant capacity (TAC), and vitamin C concentration in their blood compared to CO cows. Vitamin C supplementation had no effect on plasma iron, copper, and zinc concentrations of PP cows. Similarly, vitamin C supplementation had no effect on total feed intake and feed conversion ratio (FCR) of suckling calves. However, calves in the CMVC group had higher ($P < 0.05$) overall daily weight gain compared to the other groups. Calves in the CVC and CMVC groups had lower ($P < 0.05$) blood MDA concentration on days 7 and 21. The highest ($P < 0.05$) blood TAC level was recorded in CMVC calves. Control group calves had lower ($P < 0.05$) blood superoxide dismutase activity compared to the other calves. Blood levels of alanine aminotransferase on days 7 and 21 and aspartate aminotransferase on day 7 were higher ($P < 0.05$) for calves in the CON and MVC groups. Based on the results, vitamin C supplementation had positive health effects on the oxidative parameters of PP dairy cows and also improved the performance and health status of the calves.

**Keywords** Blood metabolites · Pre-parturient cows · Growth performance · Newborn calves · Vitamin C

## Introduction

The transition from pregnancy to early lactation in dairy cows is the most critical period in the lactation cycle and is known as the transition period. The transition period begins about 3 weeks before parturition and lasts up to 3 weeks after parturition, and is characterized by dry matter reduction, negative energy balance, high incidence of metabolic disorders, and immune system malfunction (Spicer et al. 1993). The considerable increase in nutrient requirements of pre-parturient cows (PP cows) due to fetal growth and the

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Published online: 14 January 2022

onset of milk production coincided with increased oxygen demand which results in increased production of reactive oxygen species (ROSs). Increased ROSs production and their accumulation in the tissues result in a condition known as oxidative stress in PP cows. Oxidative stress occurs when the balance between the production of ROSs and the availability of the antioxidants responsible to remove them is impaired (Sordillo and Aitken 2009). Pre-parturient cows encounter a variety of stressors including metabolic stress, nutritional stress, stress of parturition and lactation onset, and stress from different infectious and non-infectious diseases resulting in increased ROSs production. Available antioxidants decrease during pre-parturient period due to increased usage to scavenge ROSs and decreased supply of new dietary antioxidants due to reduced dry matter intake (Spears and Weiss 2008; Trevisi and Minuti 2018). Oxidative stress is an important factor in dysfunctional immune responses in metabolically stressed PP cows (Sordillo et al. 2009). Therefore, it is important for PP cows to manage and remove accumulated ROSs to ensure a functional immune system. A complex network of different antioxidants, including some vitamins, minerals, proteins, and enzymes, works synergically with each other to prevent the production of new ROSs and remove existing ROSs from cells and tissues (Sordillo 2016).

Vitamin C is the most abundant and probably the most important water-soluble antioxidant in mammals with a significant effect in decreasing oxidative stress (Ghanem et al. 2008). Vitamin C in cows is principally synthesized in the liver and dietary vitamin C, in the form of ascorbic acid, is mostly degraded by rumen microorganisms (Nockels 1988). Although it has been established that ruminants do not have any requirement for dietary vitamin C, however, ruminants may be more susceptible to vitamin C deficiency when liver function is impaired and stressful situations occur (Padilla et al. 2006). Impaired liver function is a common occurrence in PP cows due to triglyceride accumulation in hepatocytes, extensive nutrient metabolism, and liver-related metabolic disorders such as fatty liver and ketosis (Drackley 1999; Trevisi and Minuti 2018). Previous studies have conclusively documented a decrease in vitamin C level in the blood of lactating cows (Weiss et al. 2004) and calves (Cummins and Brunner 1991) that were exposed to stress and diseases. In addition, supplementation of vitamin C resulted in reduced severity of clinical signs of mastitis (Chaiyotwittayakun et al. 2002) and reduced milk somatic cell count (Weiss and Hogan 2007). The concentration of vitamin C in inactivated human neutrophils is high but increases up to tenfold in activated ones (Washko et al. 1993) which may be a response mechanism to protect cells from ROSs produced by neutrophils (Weiss et al. 2004). In addition, production of endogenous vitamin C was not observed in dairy calves until 4 months of age, and therefore, suckling calves were dependent on the moderately low vitamin C in the milk (Hidiroglou et al. 1995). Therefore, supplementation of vitamin C can be considered a significant source of antioxidants for stressed ruminants such as PP cows and newborn calves. Rumen microorganisms tend to quickly degrade vitamin C (ascorbic acid, Padilla et al. 2007); however, the half-life of polyphosphorylated ascorbic acid in the rumen was higher than ascorbic acid making it a more suitable vitamin C supplement (6.9 h versus 3.5 h, respectively) (Macleod et al. 1999). Several studies have shown that dietary supplementation of polyphosphorylated ascorbic acid was effective in increasing plasma concentrations of vitamin C in dairy cows (Hidiroglou 1999; Macleod et al. 1999; Weiss 2001; Weiss and Hogan 2007). Although there are some studies on the effects of vitamin C supplementation in PP cows (Weiss and Hogan 2007) or in suckling calves (Hidiroglou et al. 1995), there is no integrated study focusing on the effects of vitamin C supplementation on PP cows and their calves.

Therefore, the aim of the present study was to investigate the effects of dietary vitamin C supplementation on blood metabolites, blood mineral, and vitamin C concentrations and growth performance of PP dairy cows and calves.

## Material and methods

### Animals and diets

Forty PP Holstein cows (average weight = 791 ± 50 kg; 20 cows with parity 2 and 20 cows with parity 3) were selected from the Moghan Agro-Industrial and Animal Husbandry dairy herd (Parsabad, Ardabil province, Iran). The study began in early December and lasted until late February 2018. The PP cows were blocked for body weight and parity and were randomly assigned to the experimental treatments approximately 21 days before expected calving day. The experimental treatments were (1) basal diet without vitamin C supplementation (CO) ($n = 20$) and (2) basal diet with 20 g per day of vitamin C (VC20) ($n = 20$). After calving, the experiment continued by grouping the calves into four experimental groups. Thirty-two newly born Holstein calves (average weight 39 ± 0.80 kg) were selected and allocated to four treatments: (1) control calves with no vitamin C supplementation and from cows that received no vitamin C supplement (CON), (2) calves supplemented with 600 mg of vitamin C per day and from cows that received no vitamin C supplement (CVC), (3) calves supplemented with no vitamin C and from cows that received 20 g of vitamin C per day (MVC), and (4) calves supplemented with 600 mg of vitamin C per day and from cows that received 20 g of vitamin C per day (CMVC). The vitamin C supplement used in this study was Rovimix Stay-C (sodium and calcium

ascorbyl-2-phosphate). Diets of PP cows were formulated based on NRC (2001) recommendations for a dry cow (last 21 days of pregnancy) (Table 1). Isolation of newborn calves was performed immediately after birth, and after weighing, they were transferred to individual pens bedded with wheat straw. The experiment started from birth until they were weaned at day 65. Calves were given colostrum (6 L) for 2 to 8 h after birth and for the first 2 days of life. Thereafter, calves were fed whole milk twice a day (at 08:30 and 18:00 h) based on the milk feeding program of the herd. Guidelines and manuals of the animal welfare and protection council of Iran (1995) were used for all animal procedures in carrying out the study.

## Sampling and analysis

Feed samples were air-dried at 60 °C, ground with a laboratory mill (1 mm sieve), and analyzed for crude protein, ether extract (AOAC 1990), neutral detergent fiber, and acid detergent fiber (Van Soest et al. 1991). Measurements of calves' body weight (BW) changes were performed at birth and days 30 and 65 before the morning feed. Differences between feed offered and orts were assessed as the amount of individual starter intake every 2 days.

Blood collection was performed via jugular venipuncture (18-gauge × 3.8-cm needle) into 3 individual vacuum tubes: a 10-mL vacuum tube without anticoagulant for serum collection, a 8-mL heparinized vacuum tube for plasma collection, and a 5-mL tube for whole blood collection. Serum was harvested following centrifugation at 2000 × *g* for 15 min at 4 °C and plasma was harvested after centrifugation at 1000 × *g* for 10 min at 4 °C. Serum and plasma samples were stored at − 80 °C until analysis. Blood samples were collected from cows on 21 days before the expected calving day and on the calving date. Blood samples were also collected from the calves on days 2, 7, and 21. Blood samples were analyzed for glucose, cholesterol, triglyceride, albumin, total protein, HDL, LDL, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) using commercial kits (Pars Azmoon, Tehran, Iran). The activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured on whole blood samples using Ransod and Ransel kits, respectively (Randox Laboratories, UK). Measurement of total antioxidant capacity (TAC) was done in serum samples according to total antioxidant status assay (Randox Laboratories, UK). Serum malondialdehyde (MDA) content was determined according to the method described by Moore and Roberts (1998). Serum copper, zinc, and iron concentrations were determined using an atomic absorption spectrophotometer (Varian SpectrAA220, Australia). For vitamin C determination, 300 µL of plasma samples were mixed with 60 µL of 9 g/L dithioerythritol (DTE), vortexed, and kept at − 20 °C until analysis. On the day of analysis, samples were subjected to a threefold dilution with ice-cold DTE in methanol (1 g/L) and centrifuged at 10,000 × *g* for 2 min and sent to a pathobiology laboratory and analyzed by high-performance liquid chromatography (HPLC) system set up for vitamin C determination (Noor pathobiology and genetic laboratory, Tehran, Iran).

**Table 1** Ingredients and chemical analysis of pre-parturition and starter diets

| | Pre-parturition diet | Starter diet | |
|---|---|---|---|
| Alfalfa hay | 20.06 | - | |
| Corn silage | 51.46 | - | |
| Wheat straw | 15.44 | - | |
| Beet pulp | 2.05 | | - |
| Corn grain | 2.12 | | 41.5 |
| Barley grain | 1.28 | | 12.0 |
| Wheat bran | 3.60 | | 5.0 |
| Cottonseed meal | 3.60 | | - |
| Soybean meal | - | | 38.6 |
| Salt | - | | 0.4 |
| Magnesium oxide | 0.15 | | - |
| Sodium bicarbonate | - | | 0.5 |
| Shelf powder | - | | 1.0 |
| Vitamin premix | 0.15 | | 0.5 |
| Mineral premix | 0.11 | | 0.5 |
| Chemical composition | | | |
| Crude protein (% DM) | 13.0 | 19.70 | |
| Neutral detergent fiber (% DM) | 50.06 | 16.25 | |
| Acid detergent fiber (% DM) | 31.7 | 7.31 | |
| Ethereal extract (% DM) | 2.64 | 2.26 | |

## Statistical analysis

Data collected over time such as feed intake, daily gain, and feed conversion ratio were analyzed as a completely randomized block design using repeated measurement analysis by the MIXED procedure of SAS statistical software (2003). Cows were blocked based on their parity into two blocks (parity 2 and parity 3). The statistical model of the PP cows study included the effect of treatment, time, and their interactions and parity (block) as the fixed effects and the effect of calves within treatment and residual error as the random effects as follows:

$$Y_{ijkle} = \mu + A_i + AT_{ij} + B_k + R_l + E_{ijkle}$$

where:

$Y_{ijkle}$: the dependent variable, $\mu$: the overall mean, $A_i$: effect of vitamin C supplementation, $T_j$: effect of time, $AT_{ij}$: interaction effect of treatment by time, $B_k$: the effect of parity

as the block, $R_l$: random effect of calves within the treatment, and $E_{ijkle}$: the residual error. Blood data were analyzed as a completely randomized design using the GLM procedure of SAS (SAS 2003) with the effect of vitamin C supplementation as treatment effect. The model for calf study included the birth weight of calves as the covariate without the effect of parity.

The procedure of least squares means, and Tukey–Kramer test were used to describe treatments and to indicate significant differences among the treatments. Significant differences were considered at $P \leq 0.05$ and a significant trend was declared at $0.05 < P \leq 0.10$.

## Results and discussion

Supplementation of PP cows with vitamin C did not affect blood concentrations of glucose, cholesterol, triglyceride, total protein, and albumin, and blood activities of alanine aminotransferase (ALT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) (Table 2). However, PP cows who received 20 g of vitamin C per day (VC20) significantly had lower blood concentration of malondialdehyde (MDA) and aspartate aminotransferase (AST) activity and higher total antioxidant capacity (TAC) compared to the control group ($P < 0.05$). Blood mineral concentrations (copper, zinc, and iron) were not affected by vitamin C supplementation whereas PP cows in VC20 dietary group had higher ($P < 0.05$) blood vitamin C concentration compared to CO cows (Table 3).

Vitamin C or ascorbic acid is the most important antioxidant based on its effects on scavenging free radicals and regenerating other antioxidants like vitamin E (Sordillo 2016). Even though vitamin C is not an essential nutrient for adult ruminants due to its synthesis in the liver, however, dietary or parenteral supplementation of VC may be required when there is a significant increase in the production of reactive oxygen species (ROSs) due to oxidative stress, liver function impairment, or disease incidence. Pre-parturient cows typically experience all of these situations during their last month of pregnancy so vitamin C supplementation may be beneficial for these cows. Pre-parturition period is the most stressful period of the pregnancy-lactation cycle of a dairy cow because of the effects of different metabolic, oxidative, and infectious stressors (Padilla et al. 2006; Weiss and Hogan 2007). In the present study, vitamin C supplementation of PP cows increased blood vitamin C concentration that indicates the effectiveness of ascorbyl-2-phosphate as a vitamin C supplement for adult ruminants and this observation is consistent with earlier reports (Tyler and Cummins 2003; Weiss and Hogan 2007). Vitamin C plays an important role in maintaining the redox state of cells and scavenging other oxidized biomolecules and therefore increases the total antioxidant capacity of cells (Sordillo and Aitken 2009). This explains the higher TAC level and lower MDA concentration in PP cows supplemented with vitamin C compared to control cows. Malondialdehyde is a product of lipid peroxidation in tissues and as a biomarker indicates the peroxidation of poly unsaturated fatty acids and the existence of oxidative stress (Asghari et al. 2021). Although vitamin C is a water-soluble antioxidant, however, it may play a key role in ameliorating the effects of lipid-soluble ROSs duo to its vital role in converting the oxidized form of vitamin E to its reduced form (Sordillo 2016). Blood levels of liver enzymes including ALT and

**Table 2** Effect of vitamin C supplementation on blood parameters of pre-parturient dairy cows

| | Experimental treatment | | *P*-value | SEM |
|---|---|---|---|---|
| | CO | VC20 | | |
| Glucose (mg/dL) | 73.11 | 75.75 | 0.12 | 0.95 |
| Cholesterol (mg/dL) | 84.12 | 84.62 | 0.95 | 4.93 |
| Triglyceride (g/dL) | 24.37 | 23.62 | 0.84 | 0.89 |
| HDL (mg/dL) | 56.62 | 58.37 | 0.59 | 2.53 |
| LDL (mg/dL) | 4.50 | 3.87 | 0.41 | 1.03 |
| Total protein (g/dL) | 7.26 | 6.92 | 0.07 | 0.13 |
| Albumin (g/dL) | 3.22 | 3.10 | 0.15 | 0.05 |
| Superoxide dismutase (U/g) | 38.91 | 37.66 | 0.71 | 1.20 |
| Glutathione peroxidase (U/g) | 587.09 | 620.71 | 0.67 | 50.17 |
| Malondialdehyde (µmol/L) | 2.54[a] | 2.11[b] | 0.01 | 0.08 |
| Total antioxidant activity (µmol/L) | 0.396[b] | 0.442[a] | 0.003 | 0.01 |
| Alanine aminotransferase (U/L) | 24.44 | 24.00 | 0.64 | 0.63 |
| Aspartate aminotransferase (U/L) | 54.00[a] | 47.85[b] | 0.05 | 2.12 |

CO = pre-parturient cows without vitamin C supplementation, VC20 = pre-parturient cows received 20 gr per day vitamin C

[a,b,c]Means in the same row with different superscripts are significantly different ($p < .05$)

**Table 3** Effect of vitamin C supplementation on blood concentrations of zinc, iron, copper and vitamin C of pre-parturient dairy cows

| | Experimental treatments | | *P*-value | SEM |
|---|---|---|---|---|
| | CO | VC20 | | |
| Zinc (µg/dL) | 66.55 | 61.55 | 0.35 | 3.66 |
| Copper (µg/dL) | 34.50 | 39.92 | 0.39 | 3.19 |
| Iron (µg/dL) | 138.90 | 141.14 | 0.85 | 7.81 |
| Vitamin C (µg/mL) | 9.05[b] | 12.14[a] | 0.003 | 0.58 |

CO = pre-parturient cows without vitamin C supplementation, VC20 = pre-parturient cows received 20 gr per day vitamin C

[a,b,c]Means in the same row with different superscripts are significantly different ($p < .05$)

AST are indicator of liver integrity and their higher levels in the blood indicate damage to hepatocytes and therefore impaired liver function. Oxidative stress is one of the factors that may damage hepatocytes by peroxidation of their cell wall fatty acids (Asghari et al. 2021). Decreased blood level of AST observed for vitamin C supplemented cows can be attributed to increased antioxidant capacity could have alleviated the effects of oxidative stress on liver cells.

Vitamin C supplementation had significant ($P < 0.05$) effect on final body weight and average daily gain (ADG) of calves (Table 4). Calves supplemented with vitamin C and from vitamin C supplemented PP cows (CMVC) had higher ($P < 0.05$) ADG than other calves. Additionally, unsupplemented calves from vitamin C supplemented cows (MVC) had higher ADG compared to CO calves ($P < 0.05$). No significant differences were noted for feed intake and feed conversion ratio of calves. Blood activity of SOD enzyme was not affected by vitamin C supplementation on days 2 and 7 but a significant effect was observed on day 21. Calves supplemented with vitamin C (CVC), unsupplemented calves from supplemented cows (MVC), and supplemented calves from supplemented cows (CMVC) had higher ($P < 0.05$) SOD compared to the control group (CON) (Table 5). Supplementation with vitamin C had no effect on blood GPx activity of calves. On days 7 and 21, blood MDA concentration was lower ($P < 0.05$) in CVC and CMVC calves compared to MVC and CON calves. Vitamin C supplementation of suckling calves had significant effect on blood TAC on days 7 and 21, and the highest ($P < 0.05$) blood TAC was noted in CMVC calves. Level of ALT enzyme was affected by vitamin C supplementation on days 7 and 21 and not on day 2 and lower ($P < 0.05$) levels were recorded for CVC and CMVC calves. Similarly, calves in the CVC and CMVC groups had lower ($P < 0.05$) AST level compared to CON and MVC calves on day 7. Blood activity of AST was not affected by vitamin C supplementation on days 2 and 21. Vitamin C supplementation had no effect on blood zinc and iron concentrations on days 2, 7, or 21 (Table 6). Blood copper concentration was only affected on day 7 with the lowest ($P < 0.05$) concentration noted for calves in the CMVC group. Blood vitamin C concentration was not affected by vitamin C supplementation on days 2 and 7 whereas on day 21, there was a tendency for increased ($P = 0.08$) blood vitamin C concentration in the CMVC calves.

The main strategy to decrease the risk of ROSs and oxidative stress in neonatal calves is either through maternal or calf supplementation with antioxidants (Abuelo et al. 2019). Improved growth performance by supplementing antioxidants is commonly attributed to their effects on oxidative stress, immune function, and health of calves (Asghari et al. 2021). In the present study, vitamin C supplementation resulted in increased average daily gain. There is evidence that metabolic and oxidative stresses of PP cows start several weeks before calving and therefore can have significant effect on the fetus. It has been reported that stresses such as heat stress and stress of restricted or excessive energy intake during pre-parturient period will affect immune and metabolic function of calves (Osorio et al. 2013; Monteiro et al. 2016; Tao et al. 2014). Human studies revealed that suboptimal intrauterine conditions during critical periods of development may have long-term effects on physiology and disease susceptibility of babies (Merlot et al. 2008). Therefore, prenatal conditions have potential and significant effects on productivity and health of replacement heifers (Abuelo et al. 2019). This may explain why supplementing mothers with vitamin C can affect the growth performance of their calves. Ling et al. (2018) reported that calves born from cows with higher ROSs had lower body weight at birth and throughout the study. Moreover, they reported that calves exposed to higher maternal ROSs had higher serum concentrations of ROSs (Ling et al. 2018).

Minerals such as copper, zinc, selenium, and iron are part of the antioxidant system, and they function closely with other antioxidants such as vitamins. Trace minerals are

**Table 4** Effect of vitamin C supplementation on growth performance of suckling calves

| | Experimental treatments | | | | | |
|---|---|---|---|---|---|---|
| | CON | CVC | MVC | CMVC | *P*-value | SEM |
| Initial body weight (kg) | 36.86 | 36.74 | 36.00 | 37.33 | 0.70 | 0.80 |
| Final body weight (kg) | 67.16$^{c}$ | 72.00$^{b}$ | 70.33$^{b}$ | 75.16$^{a}$ | 0.001 | 0.90 |
| Average daily gain (g/day) | 500.56$^{c}$ | 586.11$^{ab}$ | 572.12$^{b}$ | 630.56$^{a}$ | 0.012 | 18.53 |
| Feed intake (g/day) | 583.61 | 653.49 | 590.17 | 624.73 | 0.16 | 24.06 |
| Feed convection ratio | 1.15 | 1.11 | 1.04 | 1.01 | 0.24 | 0.05 |

CON) control calves with no vitamin C supplementation and from cows that received no vitamin C supplement, CVC) calves supplemented with 600 mg vitamin C per day and from cows that received no vitamin C supplement, MVC) calves supplemented with no vitamin C and from cows that received 20 g vitamin C per day during their pre-parturient period, and CMVC) calves supplemented with 600 mg vitamin C per day and from cows that received 20 g vitamin C per day during their pre-parturient period

$^{a,b,c}$Means in the same row with different superscripts are significantly different ($P < 0.05$)

**Table 5** Effect of vitamin C supplement on blood oxidative parameters of suckling calves

| | Experimental treatments | | | | | |
|---|---|---|---|---|---|---|
| | CON | CVC | MVC | CMVC | *P*-value | SEM |
| Superoxide dismutase (U/g) | | | | | | |
| 2 days | 38.10 | 43.22 | 41.65 | 43.05 | 0.79 | 3.22 |
| 7 days | 39.00 | 43.00 | 44.40 | 46.70 | 0.36 | 2.59 |
| 21 days | 38.19$^{b}$ | 48.04$^{a}$ | 47.55$^{a}$ | 48.77$^{a}$ | 0.02 | 2.16 |
| Glutathione peroxidase (U/g) | | | | | | |
| 2 days | 455.28 | 452.40 | 475.28 | 472.00 | 0.80 | 18.27 |
| 7 days | 455.12 | 459.20 | 463.75 | 455.73 | 0.96 | 37.37 |
| 21 days | 440.08 | 458.03 | 474.56 | 488.50 | 0.23 | 15.89 |
| Malondialdehyde (µmol/L) | | | | | | |
| 2 days | 1.58 | 1.39 | 1.64 | 1.56 | 0.33 | 0.09 |
| 7 days | 2.06$^{a}$ | 1.76$^{b}$ | 2.04$^{a}$ | 1.80$^{b}$ | 0.01 | 0.06 |
| 21 days | 2.28$^{a}$ | 1.89$^{b}$ | 2.16$^{a}$ | 1.75$^{b}$ | 0.001 | 0.07 |
| Total antioxidant capacity (µmol/L) | | | | | | |
| 2 days | 0.140 | 0.132 | 0.142 | 0.150 | 0.88 | 0.015 |
| 7 days | 0.360$^{b}$ | 0.442$^{ab}$ | 0.317$^{b}$ | 0.500$^{a}$ | 0.03 | 0.03 |
| 21 days | 0.380$^{b}$ | 0.492$^{a}$ | 0.292$^{c}$ | 0.575$^{a}$ | 0.001 | 0.02 |
| Alanine aminotransferase (U/L) | | | | | | |
| 2 days | 9.00 | 9.01 | 9.25 | 9.75 | 0.90 | 0.75 |
| 7 days | 14.90$^{a}$ | 12.20$^{b}$ | 15.25$^{a}$ | 11.75$^{b}$ | 0.008 | 0.52 |
| 21 days | 19.00$^{a}$ | 13.40$^{b}$ | 17.25$^{a}$ | 13.25$^{b}$ | 0.001 | 0.72 |
| Aspartate aminotransferase (U/L) | | | | | | |
| 2 days | 26.00 | 24.40 | 23.00 | 25.25 | 0.89 | 2.72 |
| 7 days | 37.60$^{a}$ | 31.80$^{b}$ | 38.75$^{a}$ | 30.00$^{b}$ | 0.003 | 1.47 |
| 21 days | 50.00 | 53.00 | 54.00 | 49.00 | 0.94 | 5.61 |

CON) control calves with no vitamin C supplementation and from cows that received no vitamin C supplement, CVC) calves supplemented with 600 mg vitamin C per day and from cows that received no vitamin C supplement, MVC) calves supplemented with no vitamin C and from cows that received 20 g vitamin C per day during their pre-parturient period, and CMVC) calves supplemented with 600 mg vitamin C per day and from cows that received 20 g vitamin C per day during their pre-parturient period

$^{a,b,c}$Means in the same row with different superscripts are significantly different ($P < 0.05$)

required for various enzymatic actions in antioxidant defense system. Copper and zinc are involved in the antioxidant system due to their role in CU–ZN SOD. In addition, copper is part of ceruloplasmin that exhibits oxidase activity and zinc is a part of metallothionein that may scavenge hydroxide radicals (Spears and Weiss 2008). Availability of one antioxidant can affect the level of other antioxidants in the cells by its regenerating effect or by consuming ROSs. Ascorbic acid is a potent antioxidant that maintains ROSs levels in conjunction with vitamin E and glutathione. In addition, ascorbic acid affects intestinal and gastric absorption of some minerals and its regenerative and binding properties may be effective in the absorption and metabolism of minerals (Seifi et al. 1996). In the present study, vitamin C supplementation in calves from supplemented or unsupplemented cows had no effect on blood concentrations of zinc and iron, although some numerical increases were observed. Decreased copper concentration noted in the present study with vitamin C supplementation was significant between CON and CMVC calves. Decreased blood copper by vitamin C supplementation has been reported in rat (Van Den Berg and Beynen 1992) and guinea-pig (Tsuchiya and Bates 1997). Van Den Berg and Beynen (1992) reported that decreased blood copper concentration with vitamin C supplementation was due to increased copper uptake by liver hepatocytes.

## Conclusions

Increased supply of vitamin C (which functions as an antioxidant) had positive effects in PP cows and also improved the performance and health status of the calves. Vitamin C supplementation in PP cows improved their oxidative status as shown by its effects on total antioxidant capacity and blood malondealdehyde concentration as oxidative biomarkers. In addition, their calves had better growth performance and oxidative parameters that suggest maternal supplementation could be a useful tool in nutritional

**Table 6** Effect of vitamin C supplement on blood mineral and vitamin C concentrations of suckling calves

| | Experimental treatment | | | | | |
|---|---|---|---|---|---|---|
| | CON | CVC | MVC | CMVC | *P*-value | SEM |
| Zinc (µg/dL) | | | | | | |
| 2 days | 94.04 | 99.24 | 96.37 | 97.75 | 0.73 | 3.37 |
| 7 days | 82.24 | 88.26 | 86.87 | 91.50 | 0.49 | 4.00 |
| 21 days | 96.90 | 100.86 | 103.50 | 105.75 | 0.30 | 3.12 |
| Copper (µg/dL) | | | | | | |
| 2 days | 41.98 | 40.76 | 40.82 | 41.75 | 0.96 | 2.01 |
| 7 days | 60.60[a] | 58.00[ab] | 57.25[ab] | 50.82[b] | 0.05 | 2.27 |
| 21 days | 72.28 | 69.66 | 67.70 | 65.95 | 0.59 | 3.92 |
| Iron (µg/dL) | | | | | | |
| 2 days | 76.20 | 83.80 | 77.85 | 81.37 | 0.34 | 2.94 |
| 7 days | 71.40 | 74.00 | 75.45 | 74.75 | 0.91 | 4.06 |
| 21 days | 68.00 | 72.40 | 71.50 | 75.00 | 0.94 | 6.34 |
| Vitamin C (µg/mL) | | | | | | |
| 2 days | 0.28 | 0.36 | 0.30 | 0.35 | 0.88 | 0.023 |
| 7 days | 0.31 | 0.34 | 0.35 | 0.37 | 0.59 | 0.028 |
| 21 days | 0.33 | 0.35 | 0.34 | 0.40 | 0.08 | 0.035 |

CON) control calves with no vitamin C supplementation and from cows that received no vitamin C supplement, CVC) calves supplemented with 600 mg vitamin C per day and from cows that received no vitamin C supplement, MVC) calves supplemented with no vitamin C and from cows that received 20 g vitamin C per day during their pre-parturient period, and CMVC) calves supplemented with 600 mg vitamin C per day and from cows that received 20 g vitamin C per day during their pre-parturient period

[a,b,c]Means in the same row with different superscripts are significantly different ($P < 0.05$)

management programs. Although direct supplementation of vitamin C in calves had positive effects on growth and oxidative parameters, more research is needed to uncover the overall effects of maternal supplementation of vitamin C as well as other nutrients.

**Acknowledgements** The authors would like to thank Prof. Uchenna Y. Anele (*North Carolina Agricultural and Technical State University, Greensboro, NC 27411, USA*) for his suggestions and editing the English of this manuscript during the revision process. This research received no grant from any funding agency/sector.

**Author contribution** SS, JS, and HAB designed and conducted the experiment; JS, HAB, and AZMS were supervisors of the student PhD thesis and the experiment; SS, JS, HAB, MMMYE, AZMS, and RSS prepared the manuscript. UYA carried out extensive review of the manuscript. All Authors approved of the manuscript.

**Data availability** Not applicable.

**Code availability** Not applicable.

## Declarations

**Ethical statement** This study was approved by the Research Ethics Committee of University of Mohaghegh Ardabili, Iran (ID: IR.UMA.REC.1400.009).

**Consent to participate** All authors agree to participate in the current work.

**Consent for publication** All authors agree to publish the findings of the current research.

**Conflict of interest** The authors declare no competing interests.

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