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Supplementation Of Vitamin E And Vitamin C Improves Blood-Serum Indices, Oxidative Status And Growth Of Neonatal Calves

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Abstract
Antioxidant vitamins are associated with overall animal wellness, increased performance, and other economic indices. The aim of this study was to investigate the effects of lipid-soluble vitamin E and water-soluble vitamin C, individually and in-combination, on hematology, serum biochemistry, antioxidant status and growth performance of neonatal dairy calves. Twelve neonatal female Holstein Friesian calves were recruited for this study and distributed randomly into four groups, each group having (n=3) calves. Experimental treatments were T0 (control), T1 (500 mg of vitamin E), T2 (1000 mg of vitamin C) and T3 (500 mg of vitamin E and 1000 mg of vitamin C), where the vitamins were supplied to calves in milk. Blood from the jugular vein was collected from all calves on the day of birth (before any treatment), and on days 30 and 60 for analyses of hematological indices, serum biochemistry and antioxidant status. The body weights of all experimental calves were measured on days 1, 30 and 60. A combined supplementation with vitamins E and C had significant effects on white blood cells (WBCs), mean corpuscular hemoglobin concentration (MCHC) and pact cell volume (PCV), followed by individual treatment with vitamin C and vitamin E on day 60, except for PCV which, was higher at day 30 and remained unaffected at day 60. All investigated serum biochemical indices were affected by vitamin treatment in the calves, excluding creatinine. Glucose, total protein, albumin, urea, alanine aminotransferase and aspartate aminotransferase were significant in vitamin-treated groups, either individually or combined, on day 60 of the study. Cholesterol was higher in vitamin treatments on day 30 and afterwards remained unaffected. The total antioxidant activity was significantly higher in vitamin E supplemented and combined vitamin E/vitamin C supplemented groups on day 60. The highest body weight was recorded in calves supplemented with the combination of both vitamins followed by individual vitamins at days 30 and 60. The average daily weight gain was significantly higher in calves supplemented with a combination of both vitamins. In conclusion, the combined supplementation of vitamin E and vitamin C for the first two months of life had a positive impact on hematological parameters, serum biochemical indices, antioxidant status and growth performance of neonatal calves.

Keywords: *Antioxidant, Blood-serum indices, Growth rate, Neonatal calves, Vitamin C, Vitamin E.*

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I. Introduction

Calf health and growth are significant factors in cattle production all over the world (Bateman II et al. 2012; Kertz et al., 2017; Lorenz, 2021; Edwards and Renaud, 2025) [1-4]. The antioxidant and immune systems of neonatal calves are not mature, and their disease resistance is weak, which can lead to diarrhea or respiratory disease and affect later growth and performance (Chester-Jones et al., 2017) [5]. Oxidative stress is one of the major factors that compromises the immune cell capabilities of dairy calves in the first month of their life (Cuervo et al., 2021) [6]. Oxidation and the production of free radicals are an integral part of aerobic metabolism. A variety of reactive oxygen species (ROS) are produced by normal metabolic processes and by certain leukocyte populations during defense against disease (Sordillo and Aitken, 2009) [7]. An imbalance between increased production of ROS and the availability of antioxidant defenses needs to be reduced. The

accumulation of ROS may expose cows and calves to increased oxidative stress (Miller et al., 1993) [8]. Antioxidant molecules can be used to capture these ROS. Micronutrients, including vitamins, can maintain immune system functions and nutritive interventions can be applied for improving animal health (Schmidt et al., 2018; Alhussien et al., 2021; Liu et al., 2023) [9-11].

Ascorbic acid (vitamin C) is a water-soluble antioxidant with many diverse biological activities because it influences enzyme activities and numerous cellular functions. It is significantly found in endocrine tissues and leukocytes where it acts as a free radical scavenger (Cathcart, 1985; Schwager and Schulze, 1998; Mumtaz et al., 2021) [12-14]. Ruminants can synthesise their own vitamin C (Akinmoladun, 2021) [15], but dairy calves do not synthesise their own vitamin C until 4 months of age and therefore rely on relatively low concentrations of vitamin C in feed milk (Hidiroglou et al., 1995; Ranjan et al., 2012) [16, 17]. A deficiency of vitamin C may affect disease resistance in calves and cause diarrhea and pneumonia during the early months of their life (Sahinduran and Albay, 2004) [18]. Vitamin C also plays a role in α-tocopherol (vitamin E) regeneration. Vitamin E functions as an essential lipid-soluble antioxidant, which scavenges hydroperoxyl radicals in a lipid milieu. Its antioxidant properties play a major role in protecting erythrocyte membranes and nervous tissues (Traber and Stevens, 2011) [19]. Vitamin E has numerous possible health benefits in various biological functions, including metabolism, immunity and tissue longevity (Jensen and Lauridsen, 2007) [20].

Antioxidant vitamins are associated with overall animal wellness and increased performance and other economic indices. Both vitamin E and vitamin C have been studied for their effects on plasma levels of these vitamins and the immunity of dairy calves (Eicher-Pruiett et al., 1982; Hidiroglou et al., 1995) [16, 21]. Vitamin C alone has been supplemented in the diet for improvement of weight gain and blood-serum chemistry in calves (Seifi et al., 2010). Vitamin E alone has been studied for its effect on calf growth and immunity (Krueger et al., 2014) [22]. There is a close relationship between vitamin E and vitamin C, as both have antioxidative properties and both have positive impacts on the immune system. It has been recommended that there is a need for vitamin E and vitamin C supplements in modern dairy calf-rearing systems (Krueger et al., 2014; Sahinduran and Albay, 2004) [18, 22]. Our present study was designed to determine the effects of dietary supplements of vitamin E and vitamin C, individually and in combination, on hematology, serum biochemistry, antioxidative capacity and growth performance in neonatal dairy calves.

II. Material And Methods

Ethical approval

This study was approved by the Ethical Review Committee of the Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB) at the University of Balochistan, Quetta, Pakistan. The study was performed in accordance with the UK Animals (Scientific Procedures) Act (1986) and associated guidelines, and EU Directive 2010/63/EU for animal experiments.

Animal management

The study was conducted on a herd of Holstein Friesian cows at the Military Dairy Farm, Quetta in the Balochistan province of Pakistan. The cows were kept in a stall-shed housing, and were provided with corn silage, wheat straw, alfalfa and concentrates. Following parturition, the calves were weighed and moved to individual pens. During the first hour of the calf's life, 2 kg of dam colostrum was fed by bottle, and this was repeated every 12 hours for the next two days. Subsequently, herd milk was fed (2 kg twice a day) until the 60th day of the calf's life.

Study design

Twelve neonatal female Holstein Friesian calves (n=12) were distributed randomly into four groups, each group having three (n=3) calves. All groups were homogenous for age and sex, and all calves were healthy. Experimental treatments were T0 or control (milk without vitamin supplementation), T1 (milk supplemented with 500 mg of vitamin E), T2 (milk supplemented with 1000 mg of vitamin C) and T3 (milk supplemented with 500 mg of vitamin E and 1000 mg of vitamin C). Vitamin C and vitamin E were procured from Mediexcel Pharmaceuticals (Islamabad, Pakistan). Experimental treatments started from the day of birth (day 1) and continued until the 60th day after birth. For the determination of hematological and serum biochemical indices, 10-mL samples of blood were taken from the jugular vein of all calves on day 1 (before supplementation), and then on days 30 and 60 (Fig. 1). From this, 3-mL of blood was collected in anti-coagulated tubes with ethylene diamine tetra-acetic acid (EDTA) for complete blood count and 7-mL of blood in serum tubes was centrifuged at 1800 rpm for 10 minutes and kept at -20 ˚C until further analyses. The body weights of all experimental calves were measured on day 1 (before supplementation) and then on days 30 and 60. The average daily weight gain was calculated by subtracting the initial weight (day 1) from the final weight (day 60) and dividing it by the number of days of treatment (60). The health of the calves was monitored and recorded during the study period by the farm staff and a veterinarian.

Laboratory analysis

Blood samples were analyzed within an hour of collection for hematological indices of experimental animals. The hematological indices comprising leukocytes (white blood cells, WBCs), erythrocytes (red blood cells, RBCs), hematocrit (HCT), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), pact cell volume (PCV) and platelets (PLT) were determined on a Complete Blood Count Analyzer (PKL PPC 610H-VET, Paramedical, Salerno, Italy). Serum biochemistry was analyzed using commercial kits acquired from Innoline (alanine aminotransferase [ALAT/GPT], creatinine, glucose, urea, albumin, cholesterol), Mtd Diagnostics (aspartate aminotransferase [SGOT/ASAT]), DiaSys Diagnostic Systems (cholesterol, total protein) by using a semi-automated chemistry analyzer (HTI BioChem SA, HTI Diagnostics, MA, USA). Total antioxidant capacity (TAC) was analyzed using a commercial Bovine Total Antioxidant ELISA Kit (Bioassay Technology Laboratory, Shanghai, China) on a BioTek ELX800 plate reader (Agilent, CA, USA). Procedures for serum metabolites and total antioxidant capacity were performed according to the guidelines of the respective kit manufacturer.

Statistical analysis

All data were analyzed using SPSS (version 20.0, IBM Corp, Armonk, NY, USA). The differences in hematological indices, serum metabolites, TAC and body weight values between the treatment groups were analyzed by analysis of variance (ANOVA). The Duncan post hoc test was used to compare the measured variables at each sampling time among the treatment groups. All the data were expressed as the mean and the standard error of mean (±SEM) and a P value of <0.05 was considered as significant.

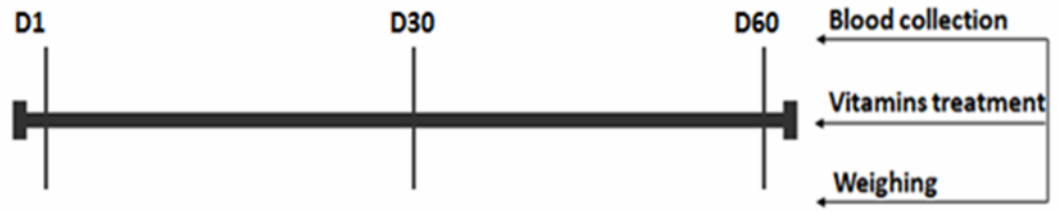

Fig. 1: Scheme of experimental design. D = number of days after birth. Blood collection and weighing of calves were conducted on days 1, 30 and 60, vitamin supplements were provided every day for 60 days.

III. Results

Hematology

The findings of hematological indices showed significantly higher (P<0.05) values of WBCs and MCHC at day 60 of vitamin supplementation. Among the supplemented groups, higher (P<0.05) values were found in that supplemented with both vitamin E and vitamin C (T3). Other hematological parameters such as RBCs, HCT, Hb, MCV, MCH, PCV and platelets were found unaffected by vitamin supplementation throughout the study (Table 1) (on page 34).

Serum biochemistry

Serum biochemical measurements from the different treatment groups are presented in Table 2 (on page 35). Glucose concentration was not significantly different between supplement and control groups from day 1 to day 30, but on day 60 the glucose was higher (P<0.05) in the vitamin C (T2) and combined supplement (vitamin C and vitamin E) (T3) groups. Total protein was significantly higher in vitamin supplemented groups on day 30 and day 60. Cholesterol was significantly higher in vitamin treated groups on day 30, whilst it was not significantly different in any group on day 60. Creatinine was not affected by vitamin supplementation. Urea was higher (P<0.05) in the vitamin E supplemented group (T1) on both day 30 and day 60, and it was also higher in the combined vitamin C/vitamin E supplemented group (T3) on day 60. Albumin, alanine aminotransferase and aspartate aminotransferase were significantly higher in groups supplemented with vitamins both individually and in combination.

Total antioxidant capacity

The TAC status of neonatal dairy calves was not affected by any vitamin supplementation, either individually or in combination, up to day 30 of the study. On day 60 TAC values were significantly higher in the vitamin E (T1) and combined vitamin C/vitamin E (T3) supplemented groups compared to control (Fig. 2).

Growth performance

There was a positive effect of vitamin E and vitamin C supplementation on growth performance (Fig. 3). The birth weight of calves was not different within the experimental groups (P>0.05). The maximum weight (P<0.05) was achieved by calves in group T3 (vitamin E and vitamin C) followed by T2 (vitamin C) then T1 (vitamin E) and T0 (control) at the end of first month (day 30) and second month (day 60) of the study. The average daily weight gain ranged from 111.66 ± 6.0 g to 275.55 ± 16.56 g in the control and T3 groups, respectively (Fig. 4). The combined supplementation of vitamin E and vitamin C (T3) resulted in the maximum average daily weight gain in the calves (P<0.05). The mortality rate was zero during the entire period of the study. The morbidity rate was not significantly different among the treatment groups during the experimental period.

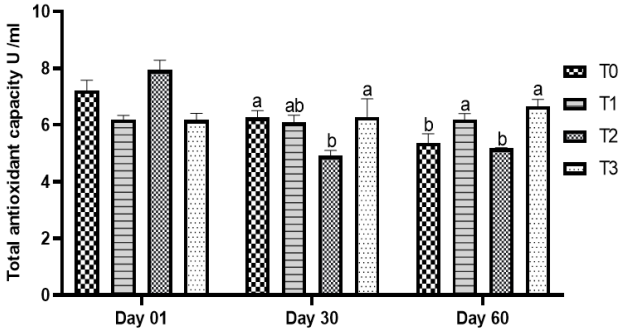

Fig. 2: Effect of vitamin E and vitamin C supplementation on total antioxidant capacity of dairy calves. T0 (control; milk without vitamin supplementation), T1 (milk supplemented with 500 mg of vitamin E), T2 (milk supplemented with 1000 mg of vitamin C), T3 (milk supplemented with 500 mg of vitamin E and 1000 mg of vitamin C). Data are presented as mean ± SEM. [a,b]Different letters show significant differences among the groups (P<0.05).

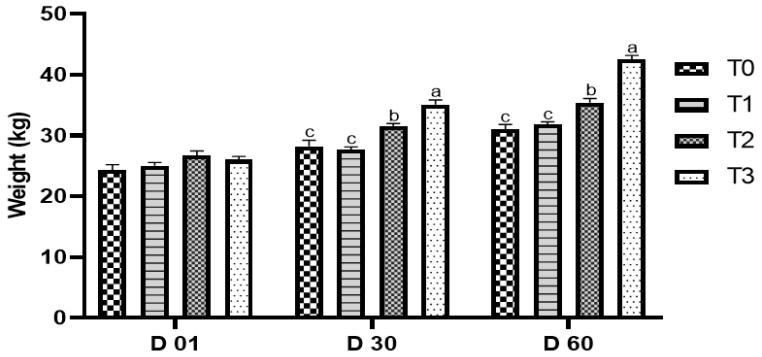

Fig. 3: Effect of vitamin E and vitamin C supplementation on the body weight of dairy calves. T0 (control; milk without vitamin supplementation), T1 (milk supplemented with 500 mg of vitamin E), T2 (milk supplemented with 1000 mg of vitamin C), T3 (milk supplemented with 500 mg of vitamin E and 1000 mg of vitamin C). Data are presented as mean ± SEM. [a,b,c]Different letters show significant differences among the groups (P<0.05).

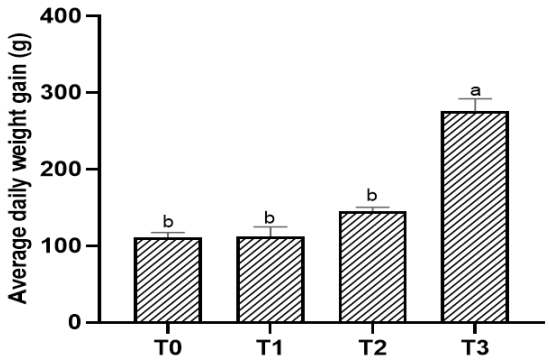

Fig. 4: Effect of vitamin E and vitamin C supplementation on the average daily gain in body weight of dairy calves. T0 (control; milk without vitamin supplementation), T1 (milk supplemented with 500 mg of

vitamin E), T2 (milk supplemented with 1000 mg of vitamin C), T3 (milk supplemented with 500 mg of vitamin E and 1000 mg of vitamin C). Data are presented as mean ± SEM. [a,b]Different letters show significant differences among the groups ($P<0.05$).

IV. Discussion

In this study, we have investigated the effects of lipid-soluble antioxidant vitamin E and water-soluble antioxidant vitamin C, individually and in combination, on hematology, serum biochemistry, TAC and growth performance of neonatal dairy calves. The combined supplementation with vitamin E and vitamin C had a greater effect on calves in terms of their blood-serum indices, TAC and growth performance.

Hematological assessment is required to evaluate the animals' performance and productivity. The hematological effects of vitamin E and vitamin C supplementation in calves are controversial. In the present study, the levels of WBC, MCHC and PCV were significantly higher with combined supplementation of vitamin E and vitamin C, while other hematological indices were not affected significantly. A previous study showed that vitamin E and vitamin C had significant effects on the values of RBC, WBC and Hb in poultry when supplemented in combination (Mbachiantim and Johnson, 2021) [23]. Another study revealed that parental administration of vitamin E and selenium exerted significant effects on WBC, RBC, Hb and PCV on the third and fourth weeks of life in dairy calves (Mohri et al., 2005) [24]. But contrary to this, another study suggested no significant effects of vitamin E supplementation on hematology in dairy calves (Schmidt et al., 2018) [9]. In a study where vitamin C was supplemented to growing dairy calves during the summer, MCV, MCH, RBC distribution width and mean platelet volume were found to be higher ($p<0.05$), whilst RBC, WBC, Hb, HCT and lymphocytes were unaffected (Kim et al., 2012) [25]. Meanwhile, a different study found no significant effects of vitamin C supplementation on hematology in neonatal dairy calves (Seifi et al., 2010) [26].

In our present study, supplementations of vitamin E and vitamin C, individually and in combination, had positive impacts on serum biochemical indices in the neonatal dairy calves. Some consistent results were revealed in previous studies regarding the effects of vitamin E and C supplementation on serum biochemistry. Like our study, Kim et al. (2012) [25] reported that total protein, albumin, creatinine and ALAT were significantly higher with vitamin C supplementation, whilst Ramezani et al. (2018) [27] reported no significant effects of vitamin C on serum biochemistry of dairy calves. Vitamin E supplementation did not exert any significant effects on blood metabolites reported by Schmidt et al. (2018) [9]. Similarly, Zayed et al. (2021) [28] reported that supplementation of vitamin E and selenium did not affect total protein, albumin, cholesterol, AST, ALT or HDL in goat kids. However, supplementation of vitamin E in combination with selenium significantly affected the levels of serum total protein, albumin and globulins in newborn goat kids (Shokrollahi et al., 2013) [29]. Another study reported that vitamin E significantly increased RBCs, Hb, WBCs and differential white blood cells. Whilst vitamin E was significantly ($p<0.001$) increased in the serum, there was reduced total protein, albumin, ALAT, ASAT, HDL, cholesterol and malondialdehyde (MDA) in the serum (Zayed et al., 2021) [28]. In our study, the positive impacts on serum biochemistry may be due to the combined effects of vitamin E and vitamin C.

Numerous biochemical systems exist in cells and extracellular fluids to remove ROS and other oxidants. Antioxidant systems include molecules such as vitamin E- and C-containing enzymes that act as membrane antioxidants to maintain the integrity of phospholipids against oxidative damage and peroxidation (Di Mascio et al., 1991) [30]. Vitamin E and C, individually or in combination, significantly influence the activities of antioxidative enzymes. A combination of vitamin E and C supplementation in diets has a positive effect on reducing the activity of the oxidative enzyme lipid peroxidase and increasing the activity of the antioxidant enzyme glutathione reductase in poultry (Panda et al., 2008) [31]. A study evaluating the effects of vitamin E and C supplementation on the antioxidant status of broiler breeds revealed that supplemented groups had significantly lowered MDA levels, higher activities of superoxide dismutase (SOD) and catalase (CAT) enzymes and higher ferric reducing antioxidant power (FRAP) activities in RBCs than control (Jena et al., 2013) [32]. Even better results were achieved when vitamins were supplemented in combination than individually. In another study, the lowest MDA levels, highest blood TAC and SOD were recorded in newborn dairy calves supplemented with vitamin C (Seifzadeh et al., 2022) [33]. Vitamin E supplementation improved the oxidative status in goat kids by decreasing the level of MDA in blood (Zayed et al., 2021) [28]. These findings agree with our study in which supplementation of vitamin E and C, individually or in combination, resulted in significantly better TAC concentrations in neonatal dairy calves.

The most salient outcome of our present study was significantly improved growth performance of dairy calves. There was a significant increase in body weight when calves were supplemented with vitamin E and vitamin C in combination. This improved effect on weight gain may be due to combined effects of vitamins E and C, their antioxidative potency and improved intermediary metabolism due to enhanced tissue oxidation by vitamin C (Seifi et al., 2010) [26]. These findings are supported by previous studies showing that body weight gain was not improved in neonatal calves by supplementation of vitamin E alone (Hidiroglou et al., 1995; Mohri et al., 2005; Schmidt et al., 2018) [9, 16, 24]. However, vitamin C supplementation alone has

improved weight gain in dairy calves (Seifi et al., 2010; Seifzadeh et al., 2021; Zakariya et al., 2024) [26, 33, 34]. Also in agreement with our study, Almuayli et al. (2023) [35] showed that a combination of vitamins A, E and C and volatile essential oils (in the form of Super Immune®) may have a better effects on antioxidant status and body weight gain in suckling calves than supplementation with the individual vitamins.

V. Conclusion

The combined supplementation of vitamin E and vitamin C to dairy calves for the first two months of their life had positive effects on hematology, serum biochemistry, antioxidant status and growth performance. A wide spectrum of studies on supplementation of antioxidant vitamins in calf milk replacer is recommended to formulate a milk replacer for optimising calf health and growth performance.

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Conflict of interest

The authors do not have any conflicts of interest to declare.

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Table 1: Effects of vitamin E and vitamin C supplementation on hematological indices of neonatal calves. T0 (control; milk without vitamin supplementation), T1 (milk supplemented with 500 mg of vitamin E), T2 (milk supplemented with 1000 mg of vitamin C), T3 (milk supplemented with 500 mg of vitamin E and 1000 mg of vitamin C). ^{a,b,c}Different superscript letters in the same row show significant differences among the groups ($P<0.05$).

Hematological parameter	Sampling time (day)	T0 (control)	T1 (Vit E 500 mg)	T2 (Vit C 1000 mg)	T3 (Vit E + Vit C)
WBC (10^9/L)	1	6.37 ± 0.47	6.4 ± 0.45	6.43 ± 0.67	5.70 ± 0.82
	30	8.27 ± 0.13	8.47 ± 0.09	8.63 ± 0.46	8.03 ± 0.03
	60	11.1 ± 0.75^{b}	11.13 ± 0.54^{b}	13.5 ± 0.50^{a}	15 ± 0.36^{a}
RBC (10^{12}/L)	1	7.62 ± 0.82	8.27 ± 0.55	6.85 ± 0.08	7.04 ± 0.52
	30	7.99 ± 0.52	6.61 ± 0.42	7.23 ± 0.34	7.68 ± 0.67
	60	8.55 ± 1.02	8.03 ± 0.75	8.75 ± 0.48	7.63 ± 0.34
HCT (%)	1	34.63 ± 3.87	41.60 ± 5.62	31.53 ± 1.53	31.17 ± 2.63
	30	32.27 ± 4.18	29.40 ± 4.23	34.03 ± 3.54	34.9 ± 3.88
	60	42.77 ± 8.14	39.63 ± 5.98	40.63 ± 2.79	36.13 ± 2.23
Hb (mg/dL)	1	9.6 ± 1.32	10.73 ± 0.80	8.4 ± 0.15	8.93 ± 0.78
	30	10.23 ± 0.50	8.83 ± 0.79	9.5 ± 0.21	10.2 ± 0.99
	60	11.63 ± 1.69	10.53 ± 1.01	12.43 ± 1.82	9.73 ± 0.57
MCV (fL)	1	45.5 ± 0.75	49.97 ± 3.37	46.07 ± 1.74	44.3 ± 0.81
	30	47.73 ± 2.22	43.97 ± 1.30	46.9 ± 3.21	45.33 ± 1.15
	60	49.3 ± 4.79	48.87 ± 3.06	46.6 ± 0.80	47.37 ± 1.30
MCH (pg)	1	12.5 ± 0.44	12.93 ± 0.26	12.23 ± 0.09	12.6 ± 0.20
	30	12.8 ± 0.26	13.27 ± 0.52	13.1 ± 0.35	13.23 ± 0.12
	60	13.47 ± 0.50	13.07 ± 0.15	12.63 ± 0.23	12.67 ± 0.20
MCHC (mg/dL)	1	34.17 ± 2.18	33.33 ± 2.32	36.67 ± 0.79	32.97 ± 3.08
	30	35.8 ± 0.90	33.77 ± 2.09	32.63 ± 1.26	32.57 ± 2.83
	60	30 ± 1.76b^{b}	30.4 ± 3.06^{b}	27.93 ± 0.41^{c}	36.9 ± 0.89^{a}
PLT (10^9/L)	1	417.33 ± 10	348.33 ± 39	359 ± 70.0	586.67 ± 42
	30	348.3 ± 14.75	382.33 ± 76.36	487.67 ± 76.49	444.67 ± 41.79
	60	492 ± 27.47	401.67 ± 56.30	358.67 ± 38.23	527.33 ± 64.38
PCV (%)	1	0.35 ± 0.14	0.27 ± 0.01	0.26 ± 0.05	0.53 ± 0.10
	30	0.27 ± 0.02	0.4 ± 0.16	0.51 ± 0.12	0.6 ± 0.31
	60	0.44 ± 0.06^{a}	0.29 ± 0.03^{ab}	0.26 ± 0.02^{b}	0.42 ± 0.06^{ab}

Table 2. Effects of vitamin E and vitamin C supplementation on serum metabolites of neonatal calves. T0 (control; milk without vitamin supplementation), T1 (milk supplemented with 500 mg of vitamin E), T2 (milk supplemented with 1000 mg of vitamin C), T3 (milk supplemented with 500 mg of vitamin E and 1000 mg of vitamin C). [a,b,c]Different superscript letters in the same row show significant differences among the groups ($P<0.05$).

Serum metabolite	Sampling time (day)	T0 (control)	T1 (Vit E 500 mg)	T2 (Vit C 1000 mg)	T3 (Vit E + Vit C)
Glucose (mg/dl)	1	75.33 ± 1.71^{a}	70.7 ± 0.98^{ab}	66.77 ± 1.74^{b}	69.60 ± 3.67^{ab}
	30	63.9 ± 2.58^{a}	55.2 ± 2.18^{b}	61.3 ± 1.47^{ab}	57.63 ± 1.22^{ab}
	60	51.23 ± 0.94^{c}	58.07 ± 2.6^{b}	59.53 ± 1.27^{ab}	67.97 ± 0.81^{a}
Total protein (g/L)	1	96.17 ± 1.56^{ab}	90.63 ± 2.42^{b}	93.87 ± 2.37^{ab}	98.37 ± 2.31^{a}
	30	98.57 ± 1.65^{c}	103.7 ± 1.3^{bc}	105.43 ± 1.65^{b}	113.2 ± 2.4^{a}
	60	105.6 ± 0.35^{c}	110.87 ± 2.36^{b}	116.97 ± 0.67^{a}	118.17 ± 0.99^{a}
Cholesterol (mmol/L)	1	2.47 ± 0.38^{b}	3.7 ± 0.26^{a}	4.4 ± 0.15^{a}	4.2 ± 0.06^{a}
	30	3.40 ± 0.32^{b}	5.27 ± 0.12^{a}	5.50 ± 0.21^{a}	5.57 ± 0.2^{a}
	60	5.67 ± 0.19	5.07 ± 0.17	5.20 ± 0.15	5.53 ± 0.22
Creatanine (mg/dl)	1	0.66 ± 0.12^{c}	0.53 ± 0.03^{c}	1.13 ± 0.03^{b}	1.43 ± 0.12^{a}
	30	1.73 ± 0.07	1.7 ± 0.06	1.73 ± 0.12	1.53 ± 0.15
	60	1.67 ± 0.19	1.60 ± 0.06	1.67 ± 0.12	1.83 ± 0.03
Albumin (g/L)	1	30.4 ± 0.1	31.1 ± 0.31	31.73 ± 0.39	30.97 ± 0.39
	30	33.63 ± 0.3^{ab}	34.63 ± 0.3^{a}	32.87 ± 0.23^{b}	34.5 ± 0.21^{a}
	60	29.83 ± 0.2^{b}	32.60 ± 0.68^{ab}	32.86 ± 0.23^{a}	33.73 ± 1.59^{a}
Urea (mmol/L)	1	2.53 ± 0.19^{b}	2.8 ± 0.06^{b}	3.07 ± 0.26^{b}	4.17 ± 0.18^{a}
	30	5.03 ± 0.12^{c}	7.17 ± 0.03^{a}	6.63 ± 0.13^{b}	5.13 ± 0.12^{c}
	60	6.10 ± 0.36^{c}	6.83 ± 0.09^{ab}	6.33 ± 0.09^{bc}	7.33 ± 0.15^{a}
ALAT/GPT (U/L)	1	27.8 ± 0.42	26.17 ± 1.53	24.07 ± 1.58	26.83 ± 0.2
	30	24.83 ± 0.39	25.93 ± 1.33	26.6 ± 0.42	28.3 ± 1.47
	60	30 ± 2.35^{b}	36.63 ± 0.38^{a}	34.77 ± 1.22^{a}	37.53 ± 0.28^{a}
ASAT/GOT (U/L)	1	92.97 ± 5.38	92 ± 3.1	92.53 ± 0.15	99.6 ± 2.14
	30	103.97 ± 1.58	106.6 ± 1.24	102.57 ± 1.62	104.1 ± 1.79
	60	107.77 ± 0.26^{c}	127.07 ± 3.19^{a}	115 ± 0.81^{b}	115.77 ± 0.52^{b}