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ORIGINAL PAPER

INFLUENCE OF PARENTERAL APPLICATION OF VITAMINS E, A AND BETA-CAROTENE TO PREGNANT COWS ON SELECTED PARAMETERS IN THE COWS' SERUM AND ON THE QUALITY OF COLOSTRUM*

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Abstract

The aim of the study was to determine the effect of parenteral application of two vitamin preparations to pregnant cows (vitamins E and A to the1st experimental group, and *β*-carotene to the 2nd experimental group) in the period of 10-14 days before parturition on the following selected parameters in their serum: the levels of vitamins E, A, *β*-carotene, total antioxidation status (TAS), haematological parametres, and in their colostrum: vitamins E, *β*-carotene, evaluation according to the Brix scale with a refractometer. The cows' blood samples were collected three times: 10-14 days before expected calving, on the day of calving and 7 days after calving. Colostrum samples were collected once, immediately after calving. A statistically significant difference in the *β*-carotene levels after parenteral application was observed in the 2nd experimental group (6.05 μmol l^{-1}; $P<0.01$) at the day of calving when compared to the control group (3.61 μmol l^{-1}), and 7 days postpartum (5.35 μmol l^{-1}; $P<0.001$) when compared to the control group (3.41 μmol l^{-1}). The level of TAS was significant ($P<0.001$) for this group in the second (0.51 mmol l^{-1}) and third sampling (0.88 mmol l^{-1}; $P<0.01$) when compared with the control group (0.50 mmol l^{-1}; respectively 0.75 mmol l^{-1}) and the 1st experimental group (0.44 mmol l^{-1}; respectively 0.70 mmol l^{-1}). There were no significant changes in the level of vitamin E and vitamin A in cow serum, in haematological parametres and in the quality and concetrations of vitamins in colostrum between the groups. The parenteral application of synthetic *β*-carotene had an effect on the level of *β*-carotene in the 2nd experimental group and was likely to affect also the level of TAS in this group. On the other hand, the level of vitamin E, A in serum and the quality of colostrum were not influenced by parenteral vitamin application.

Keywords: vitamin E, vitamin A, beta-carotene, serum, colostrum, antioxidant capacity.

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INTRODUCTION

The peripartum period (3 weeks before and 3 weeks after calving) is a challenging period for high-yielding dairy cows (Spears,Weiss 2008). It is considered critital as regards health, immunity, metabolism, fertility, milk production and hormonal changes (Goff, Horst 1997, Illek et al. 2009, LeBlanc 2013, Mikulková et al. 2020). In transiting cows, vitamins and antioxidants play an important role in preventing the weakening of immunity and the outbreak of diseases. Vitamins and non-enzymatic antioxidants are also an important part of the antioxidant system.

Vitamin E, presents in 8 different forms, is one of the most important components of cellular antioxidant systems in nature. Of the 8 forms, the most biologically active one is alpha-tocopherol, which is effective in protecting tissue from oxidative damage and thus suppressing or preventing the development of diseases. Its supplementation with supra-nutritional concentrations can lead to improved immune functions (Baldi 2005). Similarly to vitamin E, β-carotene is a lipid-soluble antioxidant, precursor (provitamin) of vitamin A. β-carotene may affect the immune response (Chew, Park 2004).

A study by Johansson et al. (2013) concluded that cows in organic dairy production can fulfill their requirements for vitamins A and E without supplementation. The exception is the period around calving, when the requirements for vitamins are higher than in the other periods. Also, non-supplemented cows in this study had a higher incidence of mastitis. Deficiency of vitamins, as well as minerals, occurs because of the requirements of the growing fetus, colostrum formation and the onset of lactation (Goff, Horst 1997, LeBlanc et al. 2004, Rizzo et al. 2013). However, the loss of vitamins in colostrum can be attributed not only to reduced dry matter intake during this period, but also to impaired vitamin distribution (Drackley 1999, Baldi 2005). Vitamins can be supplemented in prepartal cows, which can have an influence on the level of vitamins postpartum. Vitamin status of dairy cows can be also affect by genetic traits, biological factors, dry matter intake, time around calving (Goff, Stabel 1990, Jensen et al. 1999) and decreased concentration of lipoproteins (Grummer, Carroll 1991).

The aim of the present study was to determine whether the parenteral administration of vitamins E, A and β-carotene to pregnant cows 10-14 days before calving can affect the level of vitamins and total antioxidation status (TAS) in the serum of cows and the level of vitamins and the quality of colostrum according to the BRIX scale.

MATERIALS AND METHODS

Animals

The work was performed on a Holstein cattle farm with a number of 498 lactating cows and an average yield per lactation 10.249 litres of milk. Only clinically healthy adult cows in the first, maximum in the third lactation, with an average body condition score 3-3.5 without past metabolic or infectious diseases were selected for the experiment. The cows were housed on a deep straw mat; they had an at libitum access to water and were fed a ration for dried cows (Table 1). The cows were divided in three groups

Table 1

Composition of the total mixed ration fed to cows in kg per day per cow

Ingredients	*Antepartum*	*Postpartum*
Alfalfa hay	2	1
Barley straw	2.2	0
Concentrate (DOVP a.p.)	2.8	0
Concentrate (DOVP)	0	6
Post-extraction repessed meal	0.8	0
Palmitate	0	0.15
MP ion	0.5	0
High moisture corn	0	3
Brewers grain	0	4
Alfalfa haylage	0	6
Maize silage	15	19

DOVP a.p. – complementary feed for lactating dairy cows – *antepartum*, DOVP – complementary feed for dairy cows; MP ion – mineral supplements and protein concentrate (to prevent hypocalcaemia postpartum), mixture of anions

of 10 animals in each – the control group (without the application of any vitamin preparation), the 1st experimental group (application of a vitamin formula containing vitamins A, E and D) and the 2nd experimental group (application of a β-carotene formula). The first blood sampling (venipuncture of *vena coccygea media*) and the application of vitamins were performed 10-14 days before expected calving. Blood was collected in plastic tubes (HEMOS H-02, GAMA Group, Czech Republic) without an anticoagulant for serum determination. Blood samples for haematology examination were collected into sampling tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. The 1st experimental group was intramuscularly (gluteal muscles) applied 10 ml of ADE inj. vitamin solution. This vitamin preparation contains *Retinoli propionas* – 100 000 IU, *Ergocalciferolum* – 100 000 IU, *Tocoferoli alfa acetas* – 30 mg. Vitamin D has not been taken into account and evaluated in our work. The 2nd experimental group

received 20 ml per animal of Carofertin 10 mg ml^{-1} (*Betacarotenum* 10.00 mg) subcutaneously (praescapular area). The calving of all cows in the experiment took place spontaneously. Prompt assistance was prepared and provided if needed by controlled pull. The foetal membranes were delivered spontaneously within 12 h after calving. The second blood sampling of cows was performed immediatelly after calving. The third blood sampling was performed 5-7 days after calving.

Sampling and analyses

The samples for hematological examination were analyzed immediately after the blood collection. Anti-coagulated blood with EDTA was analyzed for hematological parametres WBC (white blood cell), RBC (red blood cell), (HGB) haemoglobin, (HCT) haematocrit, PLT (platelets) by an automatic vet hematology analyzer BC–2800 (Mindray, China).

After sampling, the serum was allowed to clot at room temperature and then separated after centrifugation at 3000 g for 10 min and subsequently stored at -70°C until analysis. The following parameters were determined in the cow serum: vitamin E, vitamin A, β-carotene and TAS. The determination of TAS was performed using standardized kits (Randox Laboratories Ltd, Crumlin, Antrim, United Kingdom) and measured by an automatic Konelab 20XT biochemical analyzer (Thermo Fisher Scientific, Vantaa, Finland).

Colostrum samples from the first milking (after calving) were collected into special plastic samplers for colostrum collecting. The quality of colostrum was checked and evaluated with a digital pocket refractometer on the Brix scale and expressed as a percentage. A Brix scale value of 22 or more indicated quality colostrum. The colostrum samples were stored at -70°C until the laboratory analysis.

The concentrations of vitamins A, E and β-carotene in the cow serum were determined using a High-Performance Liquid Chromatography (HPLC) system Ultimate 3000 (Dionex, Sunnyvale, USA) according to Sowell et al. (1994) with a minor modification. The blood samples for vitamin determination were extracted (hexane) followed by evaporation and dissolution in a mobile phase (methanol). The concentration of these vitamins in colostrum was determined by the same method according to Ubaldi et al. (2005).

Statistical analysis

The results were tested for homogeneity of variance (Hartley--Cochran-Bartlett test) and normality of distribution (Shapiro-Wilk test). The data were analysed statistically by one-way analysis of variance (ANOVA) followed by the Fisher's LSD *post hoc* test. All results were expressed as a mean value (x) ± standard deviation (SD). $P<0.05$ was considered as statistically significant.

RESULTS

The values of vitamins A, E and β-carotene in cow serum are shown in Figures 1, 2 and 3. There was no statistically significant difference in the

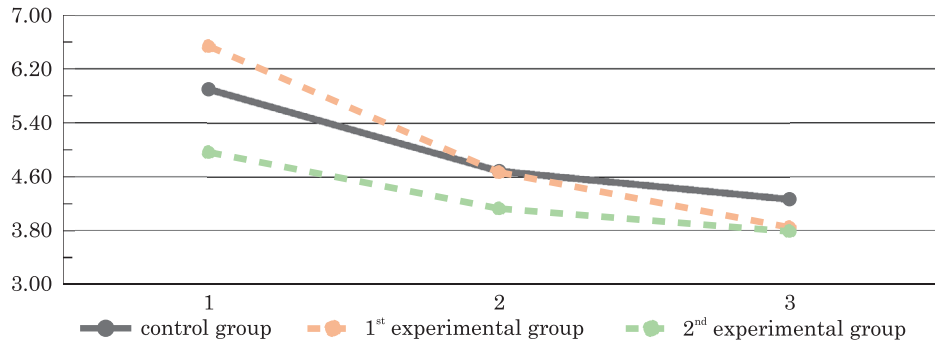

Fig. 1. The concentration of vitamin E (µmol l^{-1}) in cow serum in all samplings:
1 – first sampling, 10-14 days before calving, 2 – second sampling, the day of calving,
3 – third sampling, 5-7 days after calving

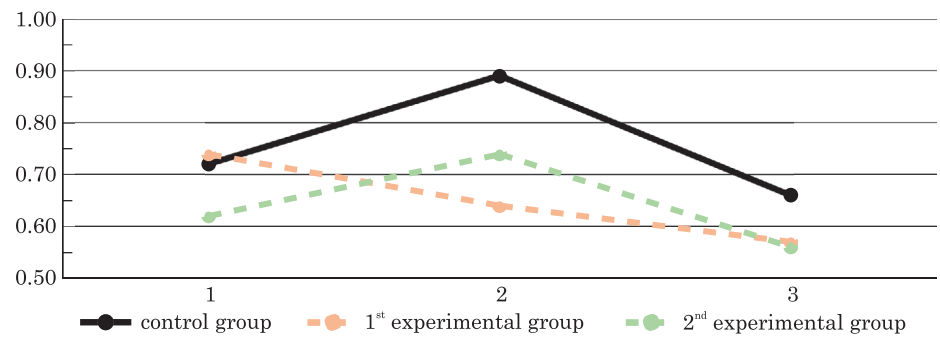

Fig. 2. The concentration of vitamin A (µmol l^{-1}) in cow serum in all samplings:
1 – first sampling, 10-14 days before calving, 2 – second sampling, the day of calving,
3 – third sampling, 5-7 days after calving

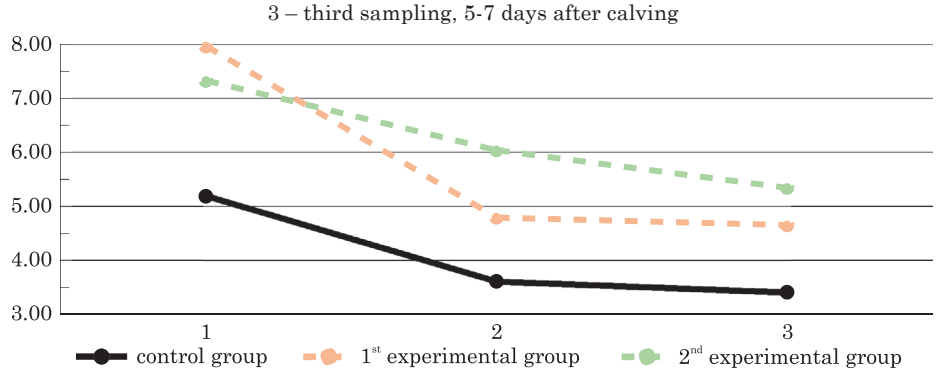

Fig. 3. The concentration of β-carotene (µmol l^{-1}) in cow serum in all samplings:
1 – first sampling, 10-14 days before calving, 2 – second sampling, the day of calving,
3 – third sampling, 5-7 days after calving

serum level of vitamin E in cows between group 1 (vitamin E), 2 (β-carotene) and the control group after the vitamin application. Only in the first sampling, the 1st experimental group showed the highest levels (6.55 μmol l^{-1}, $P<0.01$) of vitamin E compared to the control (5.91 μmol l^{-1}), and the control group ($P<0.05$) when compared with the 2nd experimental group (4.97 μmol l^{-1}). However, these levels of vitamin E decreased in the second and third sampling in all groups.

A statistically significant difference in the level of β-carotene was recorded between the 2nd experimental group (6.05 μmol l^{-1}, $P<0.01$), and the control group (3.61 μmol l^{-1}) when taken on the day of calving. A statistically highly significant difference ($P<0.001$) was observed between the 2nd experimental group (5.35 μmol l^{-1}) and the control group (3.41 μmol l^{1}) 5-7 days after calving. The level of β-carotene was also significantly higher ($P<0.01$) in this sampling in the 1st experimental group (4.66 μmol l^{-1}) compared to control group (3.41 μmol l^{-1}).

No statistically significant differences in vitamin A levels in cow serum were observed.

The determined values of vitamins E and β-carotene in colostrum are shown in Table 2. There were no significant changes in the level of vitamins

Table 2

Values of parameters in colostrum – Brix (%), vitamin E and β-carotene (μmol l^{-1})

Parameters		CO	Vit E	BETA
Brix (%)	x	25.7	26.2	22.2
	SD	5.5	5.2	6.9
Vit E (μmol l^{-1})	x	11.86	15.80	16.27
	SD	4.48	8.38	6.45
bKar (μmol l^{-1})	x	4.40	6.05	5.37
	SD	1.74	2.56	2.30

x – mean value, SD – standard deviation, no significant differences between groups were recorded $P>0.05$, CO – control group, Vit E – 1st experimental group (vitamin E), BETA – 2nd experimental group (β-carotene)

in colostrum between the groups. Although a tendency to higher vitamin concentrations was observed in the colostrum of the experimental groups. According to the Brix scale, the 1st experimental group showed the highest values (26.2 %) compared to other groups.

The level of TAS (Figure 4) was significantly the highest ($P<0.001$) in the 2nd experimental group (0.86 mmol l^{-1}) in the first sampling before the application of the vitamin preparations when compared to the control (0.74 mmol l^{-1}) and the 1st experimental group (0.63 mmol l^{-1}). Also in the second and third sampling, the level of TAS remained significantly higher ($P<0.001$) in the 2nd experimental group (0.51 and 0.88 mmol l^{-1}, respectively) compared

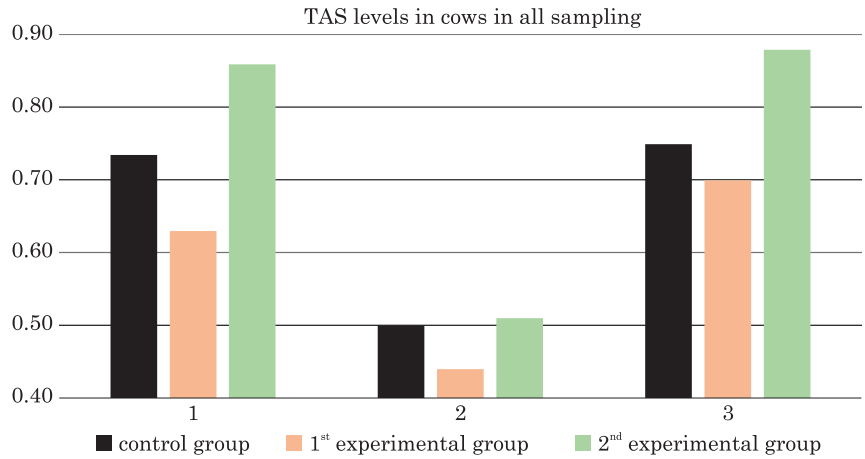

Fig. 4. The concentration of TAS (mmol l^{-1}) in cow serum in all samplings:
1 – first sampling, 10-14 days before calving, 2 – second sampling, the day of calving, 3 – third sampling, 5-7 days after calving

to the 1st (0.44 and 0.70 mmol l^{-1}, respectively) and the control group (0.50 and 0.75 mmol l^{-1}; $P<0.01$ for third sampling, respectively).

A statistically significant difference ($P<0.05$) in hematological parameters (Table 3) was observed in the control group (16.5 10^9 l^{-1}) in the WBC

Table 3

Values of hematological parameters in cows in all samplings

Parametres		10-14 days before calving			Day of calving			7 days after calving		
		CO	1st exp. group	2nd exp. group	CO	1st exp. group	2nd exp. group	CO	1st exp. group	2nd exp. group
WBC (10^9 l^{-1})	x	9.9	9.7	9.0	16.5$^{\alpha}$	13.3$^{\alpha}$	15.3	8.7	9.0	8.9
	SD	2.2	2.4	1.1	3.5	2.9	1.9	1.6	2.9	2.0
RBC (10^{12} l^{-1})	x	7.41	6.95	6.97	8.16	7.74	7.64	7.17	6.86	6.61
	SD	0.60	0.76	0.68	0.82	0.80	0.86	0.41	0.90	0.50
HGB (g l^{-1})	x	106	104	101	119	118	113	102	108	99
	SD	10	9	8	9	10	8	9	8	5
HCT (%)	x	37.1	36.6	35.9	40.9	41.8	38.8	36.1	37.5	34.8
	SD	3.5	3.2	2.9	4.2	3.3	5.5	2.9	4.7	2.0
PLT (10^9 l^{-1})	x	331$^{\alpha}$	231$^{\alpha}$	285	358	347	336	404	340	338
	SD	83	132	101	85	103	112	173	121	93

x – mean value, SD – standard deviation, significant differences between groups within a given sample are recorded using the same notation, α, β – $P<0.05$, CO – control group, 1st exp. group – 1st experimental group (vitamin E), 2nd exp. group – 2nd experimental group (β-carotene), WBC – white blood cells, RBC – red blood cells, HGB – haemoglobin, HCT – haematocrit, PLT – platelets

level compared to 1 experimental group (13.3 10^9 l^{-1}) (day of calving) and in control group in the level of PLT (331 10^9 l^{-1}) compared to 1 experimental group (231 10^9 l^{-1}) (10-14 days before calving).

DISCUSSION

It is a well-known fact that the highest need for vitamins in dairy cattle occurs around calving. This may be due to reduced feed intake, high milk production, losses in colostrum, but it may also be due to impaired vitamin distribution (Drackley 1999). When producing 10 kg of colostrum, 43 mg of retinol, 19 mg of alpha-tocopherol and 140 mg of zinc are removed from the plasma pool on the day of calving. After 3 day postpartum, plasma retinol and alpha-tocopherol levels do not decrease further or return to normal levels (Goff, Stabel 1990). Similarly, in a more recent study by Píšťková et al. (2019), a significant decrease in the level of vitamin A, vitamin E and β-carotene was observed immediately after calving.

A single intramuscular application of a vitamin preparation containing vitamins E and A had no effect on increasing the levels of these vitamins in cow serum. This may have been related to the transfer of vitamins from the blood to colostrum. During the synthesis of colostrum, a considerable amount of alpha-tocopherol is reduced from bodypools (Goff, Stabel 1990, Weiss et al. 1992). There were different results achieved by LeBlanc et al. (2002), where an injection of 3000 IU of vitamin E (RRR-aplha-tocopherol acetate) to pregnant cows 7 days before calving increased the serum alpha-tocopherol levels 3 days before calving, on the day of calving and 5-7 days after calving. Similarly, in Weiss et al. (1992), an injection of dl-alpha tocopherol (3000 IU, subcutaneously, 10 days prior to expected calving) increased the vitamin E levels 5 days before calving and on the day of calving. However, this effect was no longer detectable 7 days after calving. In the work of Sobiech et al. (2015), cows given intramuscular administration of a preparation containing tocopherol acetate and sodium selenite 5 days before calving showed an increased serum vitamin E concentration compared to the control group 5 days after calving. Vitamin E levels in cows were kept stable and high until the end of this experiment.

On the other hand, subcutaneous administration of β-carotene in the 2nd experimental group proved to be the most effective in terms of the subsequent significant increase in its serum level in cows on the day of calving and 5-7 days after calving. In studies performed by Schweigert and Eisele (1990), a single intramuscular injection of β-carotene in cows led to a significant increase in plasma concentrations of β-carotene (not retinol). The β-carotene levels remained elevated for 10 days after intramuscular administration. In the most recent study (Hye et al. 2020), cows were admini-

stered postpartum with the same β-carotene product as used in our study twice at 14-day intervals. The level of β-carotene in cows increased in the experimental group one week after the administration and reached a peak on 49 - 55 day postpartum. The TAS levels are considered an indicator of the balance between antioxidants and prooxidants. The TAS therefore evaluates the overall antioxidant capacity of the organism and provides information on total serum antioxidants (Ghiselli et al. 2001). In the research by Píšťková et al. (2019), the lowest concentration of TAS as well as the lowest concentration of vitamins A, E and β-carotene were found 2-3 days after calving. Also in our work, the level of TAS in cows decreased at the day of calving and increased at the third sampling (5-7 days after calving). The 2nd experimental group showed the highest levels of TAS in all samplings when compared to the other groups. Supplementation of calves with 20 mg β-carotene daily has been shown to be effective in reducing reactive oxygen metabolites (d-ROMs) and increasing the biological antioxidant capacity by biological antioxidant potential (BAP) test (Otomaru et al. 2018). However, in our work, the 2nd experimental group showed the highest level of TAS even before the application of the vitamin preparation itself, which could also keep its levels higher after calving.

In our study, we evaluated the quality of colostrum using the Brix scale with a digital refractometer. Digital brix refractometers (as well as the optical ones) are capable of differentiating between good and poor quality of colostrum and have an acceptable sensitivity and specifity when compared with the gold laboratory standart test, i.e. radial immunodiffusion assay. The 22% cut-off point according to the Brix scale ensures high-quality colostrum (Bielmann et al. 2010). The quality of colostrum according to the Brix scale was not significantly affected by the application of the vitamin preparation. In some other studies, too, there was no evidence of an increase in colostral IgG concentration after vitamin E (Horn et al. 2010, Moghimi-Kandelousi et al. 2020) or β-carotene (Nishijima et al. 2017) supplementation in cows. Opposite results were observed in the work of Ishida et al. (2018) after the supplementation of dry carrot in the cows' diet or in the work of Singh et al. (2013) after the vitamin E supplementation to prepartal buffalo cows.

Although the concentrations of vitamins E, A and β-carotene in colostrum of the experimental groups tended to be higher, the differences were not statistically significant. In the aforementioned work of Weiss et al. (1992), the cows of the experimental group had significantly higher levels of tocopherol in colostrum than the cows injected with placebo. In Japanese black cows, the supplementation with 500 mg per day of β-carotene in a diet 21 days before the expected calving did not affect the concentration of β-carotene in the colostrum. On the other hand, the supplementation was effective in increasing β-carotene in the plasma of these cows from calving to 60 days postpartum (Ishida et al. 2018).

A statistically significant difference in hematological parameters was observed only in the control group in the WBC level compared to 1 experimental group (day of calving) and in control group in the level of PLT compared to 1 experimental group (10-14 days before calving). In the control group, the levels of these parameters were higher. However, these significant differences are probably not related to the application of vitamin preparations. The effects of vitamin application on hematological parameters were observed in calves (Moosavian et al. 2010, Otomaru et al. 2015). In a study performed by Snarska et al. (2018), supplementation of fallow deer with vitamin E and selenium induced a prompt increase in red blood cells and hemoglobin.

CONCLUSION

A single injection of β-carotene (subcutaneously) before calving proved to be effective in significantly increasing its serum levels in cows on the day of calving as well as 5-7 days after calving. At the same time, the cows in this experimental group showed the highest serum TAS levels. On the other hand, the application of vitamins E and A (intramuscularly) had no significant effect on the increase in serum levels of these vitamins in cows, which may have indicated the consumption of these vitamins in the colostrum. Although the levels of these vitamins and β-carotene in the colostrum were shown to be higher compared to the control group, the changes were not statistically significant. Also, this treatment had no effect on the hematological parameters of the cows.

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