Effect of parenteral application of β-carotene, α-tocopherol, and selenium on selected antioxidant/oxidant parameters in dairy calves

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Abstract

The aim of this work was to determine the effect of parenteral application of vitamin preparations on selected antioxidant/oxidant parameters. Thirty Holstein heifer calves aged 7–10 days were divided into three groups: control group (no vitamin supplementation); experimental group A (application of α-tocopherol and selenium); and experimental group B (application of α-tocopherol and β-carotene). The first blood sampling and at the same time the first parenteral application of the vitamin formula in experimental groups A and B were performed in heifers at the age of 7–10 days and then again at the age of 35 days. The last blood sampling was performed at the age of 70 days. The following main parameters were determined in the blood of heifers: retinol, α-tocopherol, β-carotene, selenium (Se), glutathione peroxidase (GPx), total antioxidant capacity (TAC), and malondialdehyde (MDA). Group B showed a significantly higher concentration of β-carotene ($P < 0.001$; $P < 0.01$) and α-tocopherol ($P < 0.05$) at the second sampling compared to the control group and group A. At the third collection, a higher concentration of α-tocopherol was observed in group B ($P < 0.001$) and A ($P < 0.05$) compared to the control group. The TAC and GPx activity in calves was significantly higher in the third sampling in group A compared to the control group (for TAC; $P < 0.05$) and group B (for TAC; $P < 0.05$; GPx $P < 0.01$). Repeated parenteral administration of the vitamin preparation had a significant effect on some of the selected antioxidant parameters in calves. On the other hand, during the period of expected increased oxidative stress, a sharp decrease in most antioxidant parameters was observed.

Oxidative stress, vitamins, glutathione–peroxidase, malondialdehyde

Raising healthy calves is an essential basis for creating a healthy and prosperous herd of dairy cows. It is well known that mastering colostral immunity through the first doses of good quality colostrum is of utmost importance for the calves' life and their future health. The syndesmochorial placenta of ruminants does not allow the transfer of antibodies in sufficient quantities, and thus ruminant neonates are born dependent on the supply of antibodies, but also other essential substances (i.e. fat-soluble vitamins), from the mother's colostrum. Proper management and adherence to the established strategy starting with the management of the pre-partum cow, calving, and the well-established care for neonatal calves should be established and strictly followed (see the review by Mee 2008).

But that is not all; other challenges, such as weaning and management associated with it must be overcome to raise healthy cows. Weaning is one of the most critical periods in cattle breeding. During weaning, dairy calves are exposed to the stress of moving to shared housing, mixing groups with other calves, and changing their usual diet. Calves are particularly susceptible to oxidative stress because their antioxidant defense system is immature (Inanami et al. 1999). The work of Wolfe et al. (2023) indicated that early weaned calves have a lower immune response to abrupt weaning than later weaned calves.

The necessity of fat-soluble vitamins and trace elements with an antioxidant function for cattle is shown in the recent works of many authors, especially in terms of immunity and

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antioxidant defense (Mattioli et al. 2020; Píšťková et al. 2019), and disease prevention (Kume and Toharmat 2001; McGill et al. 2019; Strickland et al. 2021). In addition to their necessity for the calves' health, vitamins and microelements can serve as a general prevention and support in coping with the more demanding periods in the life of calves and adult cattle (Mattioli et al. 2020; Otomaru et al. 2022; Żarczyńska et al. 2024).

Therefore, supplementation of dairy cows and calves with vitamins E, A, and β-carotene and/or catalytic metals can be useful for maintaining their concentrations in the body during a more demanding period, thus ensuring all the above-mentioned facts.

In our work, two different vitamin preparations were administered parenterally: α-tocopherol in combination with selenium (Se) in one preparation, and α-tocopherol with β-carotene in the second one. The preparations were applied to experimental groups of calves between 7–10 days of age and then again at 35 days of age. The aim was to find out whether the application of the mentioned vitamin preparations could have a beneficial effect on maintaining the concentration of vitamin levels, Se, and selected antioxidant parameters. In addition, the concentrations of selected biochemical and haematological parameters in calves were determined. The secondary objective was to determine the concentration of α-tocopherol in erythrocytes and compare it with its concentration in plasma in relation to its supplementation.

Materials and Methods

Animals and study design

The experiment was conducted with 30 Holstein heifer calves on a farm with a milk yield of 10 249 litres per year. Thirty clinically healthy heifer calves were selected for the experiment and divided into three groups of 10 animals each. In the first experimental group (A), the commercial vitamin preparation Vita E Selen (Bioveta, a.s., Czech Republic; Ivanovice na Hané) was applied intramuscularly at 10 ml/100 kg of live weight (1 ml of the formula contains 25 mg tocoferoli α-acetas, and 2.2 mg natrii selenis – equivalent to 1 mg of Se). In the second experimental group (B), the vitamin preparation Dalmavital (Fatro S.P.A., Italy; Ozzano dell'Emilia), containing betacarotenum 15.00 mg, dl-α-tocopherol acetate 20 mg (18.22 mg of α-tocopherol) was applied intramuscularly at 1 ml/calf (1 ml/40 kg of live weight). The third group was the control group (C) without any vitamin supplementation. Only clinically healthy heifer calves with a minimum live body weight of 36 kg and a maximum live weight of 45 kg were selected for the experiment.

The first blood was sampled from 7- to 10-day-old calves to obtain serum and plasma for the determination of the following antioxidant parameters: retinol, α-tocopherol of plasma and erythrocytes, β-carotene, glutathione peroxidase activity (GPx), total antioxidant capacity (TAC), and Se concentration as a part of antioxidant system. Furthermore, biochemical (total protein - TP, albumin, globulin, albumin-globulin ratio A/G, aspartate aminotransferase - AST, γ-glutamyltransferase - GMT, total bilirubin - BIL, urea, creatinine - CREA and creatine kinase - CK and haptoglobin - HP), haematological (white blood cell count - WBC, lymphocytes count - Lymph, monocytes count - Mon, granulocytes - Gran, platelets - PLT, red blood cell count - RBC, haemoglobin - HGB, haematocrit - HCT), minerals (iron - Fe, magnesium - Mg) and malondialdehyde (MDA) were determined. The calves of the experimental groups were administered the mentioned vitamin preparations at the age of 7–10 days and then again at the age of 35 days of their life. The blood sample was taken at 7–10, 35 and 70 days of age.

Table 1. Milk replacer composition (selected components).

Components	Amount
Crude ash	6.9 %
Crude fibre	0.04%
Crude fat	18%
Crude protein	21%
Calcium	0.75%
Phosphorus	0.62%
Sodium	0.5%
Vitamin A	25 000 UI
Vitamin D3	5100 UI
All- rac-α-tocopherol acetate	105 mg

The calves were treated like all other calves on the farm. After birth, the umbilical cord was disinfected with iodine solution. Each calf received the first dose of mixed, good quality colostrum within 2 h after birth, and the second dose within 6 h after birth. The first dose of colostrum (3 l) was administered to the calves through an oesophageal tube according to the operating rules of the farm. The second (2 l) and subsequent doses of colostrum were fed from a bottle with a pacifier. Calves were given a starter from the 3rd day of life. The composition of the milk replacer is shown in Table 1. The calves were housed in outdoor sheds until the age of 60 days. After that, they were moved to a common stable.

The work was carried out in the operating conditions of the farm. The haematological and biochemical indices as well as Fe and Mg concentrations of the calves were monitored during the experiment as a part of monitoring health of calves.

Sampling and analysis

Blood samples from calves were collected by venipuncture of vena jugularis (maximum 10 ml per collection) in Hemos tubes (HEMOS H-02, GAMA Group, České Budějovice, Czech Republic) to determine the above mentioned indices from blood serum, plasma, and whole blood samples. After sampling, serum was allowed to clot at room temperature and then it was separated by centrifugation at 3,000 *g* for 10 min. Serum samples (for analysis of TP, albumin, AST, GMT, UREA, CREA, CK, BIL, retinol, α-tocopherol and β-carotene, TAC, haptoglobin, Fe, Mg) were frozen at −70 °C until analysis. Blood collection tubes (HEMOS H-02, GAMA Group) with heparin anticoagulant were used for determination of indices in whole blood (GPx activity, Se) and from plasma (MDA). Blood samples to obtain plasma were centrifuged (3,000 *g* for 10 min) within 2 h after collection and were frozen at −70 °C until analysis or were analysed immediately after collection. Blood samples for haematological examination and determination of α-tocopherol from erythrocytes were collected in collection tubes containing ethylenediaminetetraacetic acid (EDTA) and were analysed immediately after collection.

Haematological parameters

Blood anticoagulated with EDTA was analysed for WBC, Lymph, Mon, Gran, PLT, RBC, HGB, HCT using an automated veterinary haematology analyser BC–2800 (Mindray, Nanshan, Shenzhen, China).

Antioxidant parameters/vitamins and MDA

Total antioxidant capacity (TAC, mmol/l) was determined by a spectrophotometric method on a Konelab 20XT biochemical analyser (Thermo Fisher Scientific, Vantaa, Finland) using standardized kits from Randox Laboratories Ltd (Crumlin, Antrim, United Kingdom).

Fat soluble vitamins – retinol, β-carotene, α-tocopherol – were analysed by the High Performance Liquid Chromatography (HPLC) method, system Ultimate 3000 (Dionex, Sunnyvale, USA) according to Sowell et al. (1994) with minor modifications. Samples for the analysis of the mentioned vitamins were extracted with hexane, followed by evaporation and dissolution in methanol as a mobile phase.

The MDA concentration was determined by the HPLC system Ultimate 3000 (Dionex) after derivatization of MDA with 2, 4-dinitrophenylhydrazine (Matějčková et al. 2011).

The GPx activity (μkat/l) in whole blood was measured with a RANSEL kit (Randox Laboratories Ltd.) using the UV method according to Paglia and Valentine (1967). The above mentioned automatic Konelab 20XT biochemical analyser (Thermo Fisher Scientific) was used.

Selenium concentration (μg/l) in whole blood was analysed by HG AAS (hydride generation of atomic absorption spectrometry, SOLAAR, Thermo Scientific, Waltham, MA, USA). Prior to Se determination, the samples were prepared by mineralization with HNO_3 and H_2O_2 using a microwave digestion system (ETHOSTOUCH CONTROL, Milestone, Italy) followed by evaporation.

Preparation and analysis of samples for determination of α-tocopherol in RBC

The general preparation of samples for analysis and the analysis of α-tocopherol in RBC was performed according to Bieri et al. (1979) with minor modifications. One ml of blood was pipetted from a 2.5 ml collection tube with EDTA into a plastic tube. The RBCs were washed three times with saline. After the last wash, the saline was pipetted off and 1 ml of 0.5% pyrogallol in water was added to the RBC sediment. Subsequently, all samples were vigorously shaken and stored in a freezer at −20 °C until analysis.

After thawing the samples, 200 μl of the RBC suspension were pipetted out. A total of 600 μl of cold methane was added with slow shaking to form a fine, clump-free suspension. Subsequently, 1.2 ml of hexane was added, and the mixture was vortexed for one hour. The organic layer was dried and dissolved in the mobile phase, methanol. The concentration of tocopherol in RBC after dilution was calculated according to the following formula:

$$C_{\text{vit E in RBC}} = C_{\text{(measured concentration of vit E in RBC)}}\ \text{HCT} + 100/\text{HCT}$$

Biochemical indices and haptoglobin

The determination of TP, albumin, globulin, A/G, AST, GMT, urea, crea, CK, and BIL was performed with standardized kits supplied by commercial kits (Randox Laboratories Ltd, and BioVendor Brno, Czech Republic) on an automatic Konelab 20XT biochemical analyser (Thermo Fisher Scientific). All analyses were performed according to the manufacturer's instructions.

The concentration of haptoglobin was analysed by colorimetric assay using the mentioned automatic Konelab XT biochemical analyser (Thermo Fisher Scientific) with Haptoglobin kit (Tridelta Development Ltd., Ireland).

Other mineral elements

Serum Mg and Fe concentrations were analysed using flame atomic absorption spectrometry – FAAS (SOLAAR, Thermo Scientific, Waltham, MA, USA).

Statistical analysis

The obtained results were tested for homogeneity of variances (Hartley-Cochran-Bartlett test) and normality of distribution (Shapiro-Wilk test). The data were analysed statistically by a one-way analysis of variance

(ANOVA) followed by Fisher's LSD *post hoc* test. All results were expressed as mean value (x) ± standard deviation (SD). A P value ≤ 0.05 was considered as significant.

Results

The concentrations of vitamins

The concentrations of retinol in the calves' serum in individual samples are shown in Fig. 1. The highest concentration of retinol at the first sampling (at the age of 7–10 days, before the application of the vitamin preparation) was observed in the control group (0.47 ± 0.12 µmol/l) compared to experimental groups A (0.38 ± 0.07 µmol/l; $P < 0.05$) and B (0.32 ± 0.06 µmol/l; $P < 0.01$). However, at the second and third sampling (35 and 70 days after birth), no significant differences ($P > 0.05$) in retinol concentration were observed between the groups. Overall, the highest concentrations of retinol were observed in the third sample, i.e. 70 days of age of the calves.

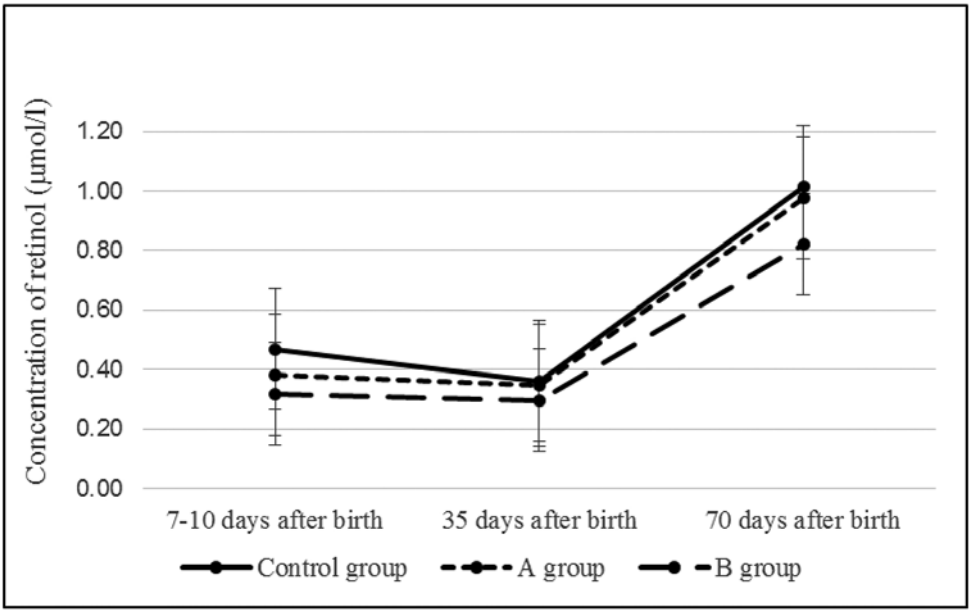

Fig. 1. Retinol concentration within groups at all three samplings

The β-carotene concentrations are shown in the Fig. 2. Although experimental group B had the lowest concentration of β-carotene at the first sampling (0.72 ± 0.33 µmol/l), it showed a significantly higher concentration at the second sampling (3.16 ± 0.83 µmol/l) compared to the control group (0.92 ± 0.56 µmol/l; $P < 0.001$) and group A (1.83 ± 1.09 µmol/l; $P < 0.01$). Nevertheless, experimental group A showed a high standard deviation. At the same time, the concentration of β-carotene was significantly higher in experimental group A ($P < 0.05$) compared to the control group. However, at the third sampling, there were no significant changes in the β-carotene concentration between the groups within each sampling ($P > 0.05$).

The concentrations of α-tocopherol are shown in Fig. 3. The concentration of α-tocopherol in the serum of calves was the highest (10.14 ± 2.73 µmol/l) in group B at the second

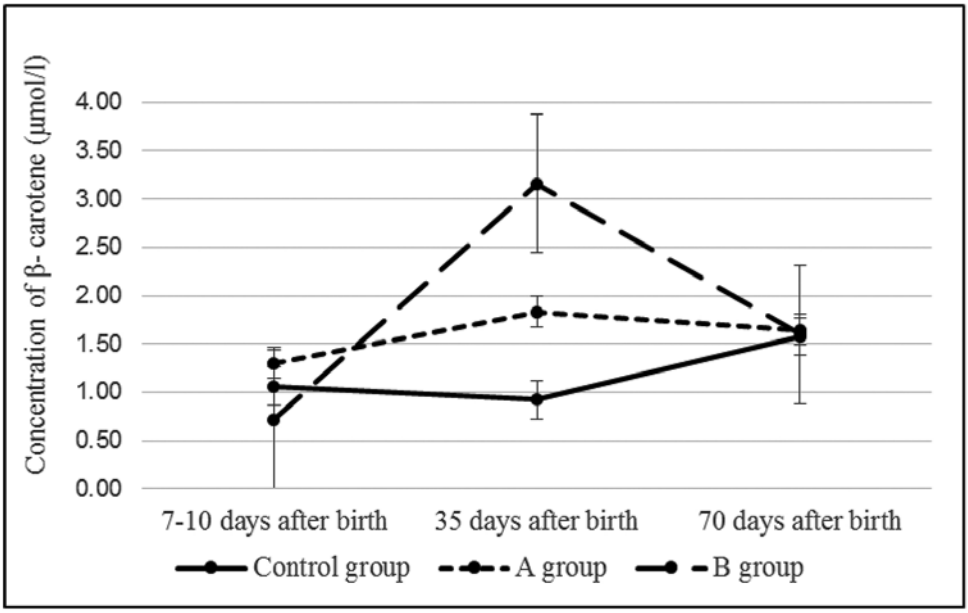

Fig. 2. Beta-carotene concentration within groups at all three samplings

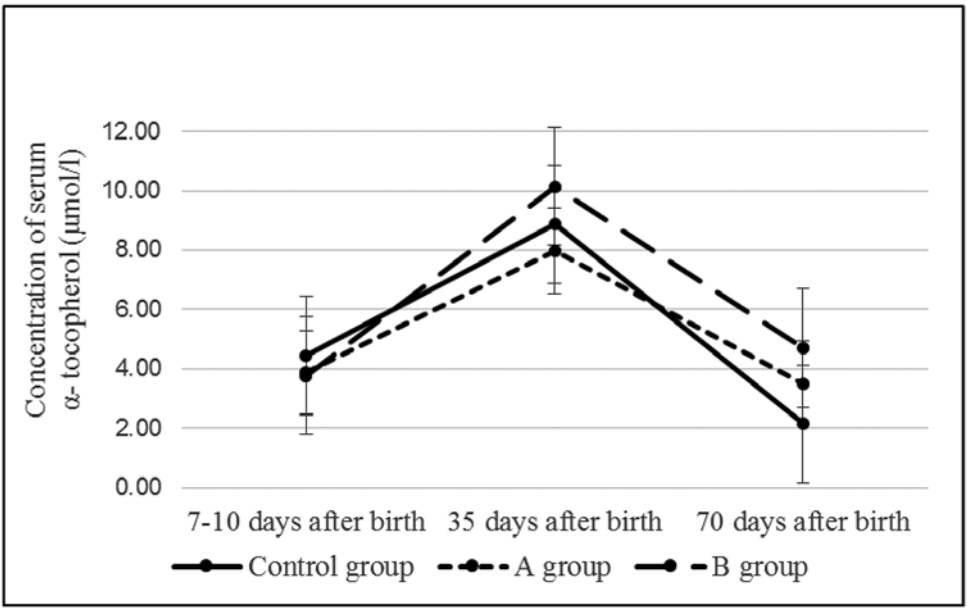

Fig. 3. Serum α-tocopherol concentration within groups at all three samplings

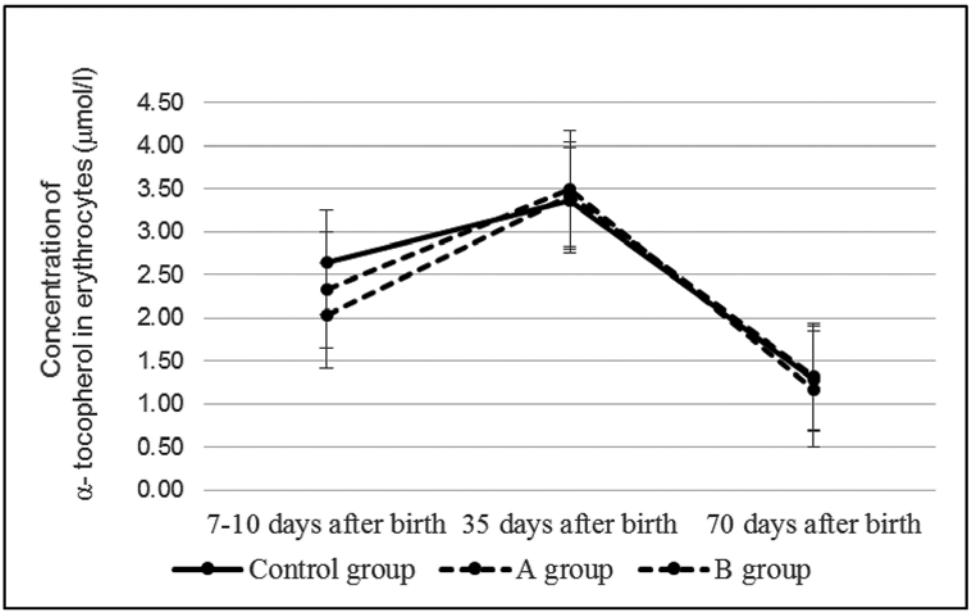

Fig. 4. Alpha-tocopherol concentration in erythrocytes within groups at all three samplings

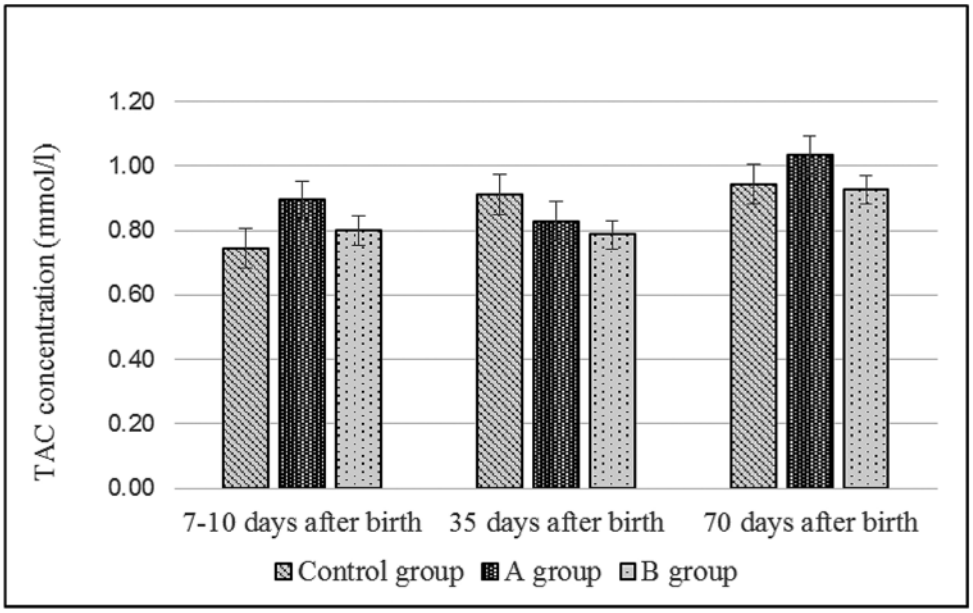

Fig. 5. Total antioxidant capacity (TAC) within groups at all three samplings

sampling after the application of the vitamin preparation, being significantly different compared to group A (7.97 ± 1.96 μmol/l; $P < 0.05$). Similarly, at the third sampling, the concentration of α-tocopherol remained the highest in group B (4.70 ± 1.28 μmol/l) compared to the control group (2.14 ± 0.58 μmol/l; $P < 0.001$) and group A (3.51 ± 1.08 μmol/l; $P < 0.01$). At the third sampling, α-tocopherol was also significantly higher ($P < 0.05$) in the experimental group A than in the control group.

There were no significant differences in vitamin E concentrations in erythrocytes between the individual groups and samplings ($P > 0.05$). The concentrations of α-tocopherols in RBC are shown in Fig. 4.

The average ratio of α-tocopherol in serum to α-tocopherol in RBC at the first sampling was as follows: control group 0.59, group A 0.52, group B 0.62; at the second sampling: control group 0.38, group A 0.43, group B 0.34; and at the third sampling: control group 0.60, group A 0.37, group B 0.25.

Concentrations of other antioxidant indices and MDA

The TAC concentrations in calves at individual samplings are shown in Fig. 5. The TAC in calves was significantly higher at the second sampling in the control group (0.91 mmol/l ± 0.113) compared to experimental groups A (0.83 ± 0.069 mmol/l; $P < 0.05$) and B (0.79 ± 0.071 mmol/l; $P < 0.01$). On the other hand, at the third sampling, group A showed the highest TAC concentration (1.03 ± 0.117 mmol/l) compared to the control group (0.95 ± 0.087 mmol/l; $P < 0.05$) and experimental group B (0.93 ± 0.069 mmol/l; $P < 0.05$).

The activity of the GPx and concentrations of Se and MDA are shown in Table 2. The GPx was significantly higher in the control group (823.43 ± 88.78 μmol/l; $P < 0.05$) compared to group B (745.01 ± 82.25 μmol/l) at the second sampling. At the third sampling, the GPx enzyme activity was significantly higher in group A (874.25 ± 91.86 μmol/l) compared to group B (755.89 ± 67.63 μmol/l; $P < 0.01$). At the same time, the GPx enzyme activity was significantly higher in the control group compared to group B ($P < 0.05$).

The Se concentration at the second sampling was significantly higher ($P < 0.001$) in the control group (173.51 ± 18.35 μg/l) compared to experimental groups A (143.73 ± 32.62 μg/l) and B (131.35 ±17.92 μg/l). At the third sampling, the highest Se concentration was observed in group A (200.83 ± 13.27 μg/l; $P < 0.001$) compared to the control group (169.37 ± 14.82 μg/l) and group B (168.32 ± 14.53 μg/l).

The MDA concentration was significantly higher in the control group (1.12 ± 0.22 μmol/l; $P < 0.01$) and in group A (1.33 ± 0.24 μmol/l; $P < 0.001$) compared to group B (0.78 ± 0.23 μmol/l) at the second sampling. In contrast, at the third sampling, MDA was

Table 2. Concentration of Se, MDA, and GPx activity in the calves' blood at individual samplings.

Indicator		7–10 days of age			35 days after birth			70 days after birth		
		Group C	Group A	Group B	Group C	Group A	Group B	Group C	Group A	Group B
GPx μkat/l	X	822.28	762.85	801.37	823.43^{α}	808.76	745.01^{α}	861.16^{α}	874.25^{a}	$755.89^{\alpha,a}$
	SD	131.53	107.59	124.03	88.78	97.03	82.25	99.62	91.86	67.63
Selenium μg/l	X	154.63	142.24	156.99	$173.51^{A,B}$	143.73^{A}	131.35^{B}	169.37^{A}	$200.83^{A,B}$	168.32^{B}
	SD	18.25	23.29	15.01	18.35	32.62	17.92	14.82	13.27	14.53
MDA μmol/l	X	1.22	1.14	1.08	1.12^{a}	1.33^{A}	$0.78^{a,A}$	$0.75^{A,a}$	1.04^{A}	0.97^{a}
	SD	0.10	0.21	0.11	0.22	0.24	0.23	0.12	0.15	0.17

X - mean value; SD - standard deviation; significant differences between groups within a given sample are recorded using the same superscript: $^{\alpha,\beta}$ - $P < 0.05$; ^{a,b} - $P < 0.01$; ^{A,B} - $P < 0.001$; Group C - control group; Group A - experimental group 1 (α-tocopherol and Se parenteral application); Group B - experimental group 2 (β-carotene and α-tocopherol parenteral application); GPx - glutathione peroxidase; MDA - malondialdehyde

the lowest in the control group (0.75 ± 0.12 μmol/l) compared to groups A and B. Significant differences ($P < 0.001$; $P < 0.01$) were observed between the control group and both groups A (1.04 ± 0.15 μmol/l) and B (0.97 ± 0.17 μmol/l).

Haematologic and biochemical indices

Concentrations of haematologic indices are listed in Table 3. The only significant difference was observed in the WBC count during the second collection (35 days of calves' age). The control group showed a significantly higher number of WBCs ($P < 0.05$) compared to group A.

Concentrations of selected biochemical elements, Fe, and Mg are shown in Table 4. The only significant difference in the haptoglobin concentration ($P < 0.05$) was observed at the second sampling between the control group (0.31 ± 0.06 g/l) and group B (0.24 ± 0.07 g/l).

Significantly the highest concentration of bilirubin at the first sampling was recorded in control compared to group A ($P < 0.001$) and B ($P < 0.01$). At the same time, the bilirubin concentration was higher in group A ($P < 0.05$) compared to group B.

Significant differences in the concentration of protein ($P < 0.05$; $P < 0.01$) and albumin ($P < 0.05$) were noted at the first (for protein) and the third sampling (for protein and albumin).

At the second sampling, significant differences in the concentration of urea ($P < 0.01$), creatinine ($P < 0.05$; $P < 0.01$), and Mg ($P < 0.001$) were noted between individual groups.

A significant difference in the concentration of protein, albumin, globulin, creatinine and GMT activity ($P < 0.05$) between groups A and B was noted at the last sampling. Also, the GMT activity at the same sampling was significantly higher ($P < 0.01$) in group B compared to control. The AST activity was significantly higher ($P < 0.01$) in the control group compared to groups A and B.

Table 3. Haematological indices in calves at different sampling times.

Indicators		7–10 days of age			35 days after birth			70 days after birth		
		Group C	Group A	Group B	Group C	Group A	Group B	Group C	Group A	Group B
WBC *10^9/l	X	9.2	9.2	9.2	10.5^{α}	8.2^{α}	9.4	11.2	9.5	9.8
	SD	2.55	2.98	2.47	3.14	1.77	1.59	3.07	1.94	2.26
Lymph *10^9/l	X	3.5	3.5	3.1	4.3	3.5	4.4	5.2	4.7	5.6
	SD	0.78	0.8	0.62	0.99	0.78	0.96	0.95	0.79	1.34
Mon *10^9/l	X	0.9	0.8	0.9	1.0^{α}	0.8^{α}	0.9	1.0	0.8	0.8
	SD	0.24	0.24	0.32	0.28	0.11	0.17	0.29	0.24	0.21
Gran *10^9/l	X	4.5	4.9	5.1	4.5	3.9	4.2	4.3	4.0	3.4
	SD	1.34	2.36	2.37	1.84	1.28	1.17	1.35	1.32	1.32
PLT *10^9/l	X	405.6	435.2	462.9	352.4	331.8	321.9	412.4	434.2	415.2
	SD	113.31	105.95	64.69	98.03	90.28	51.39	81.82	73.93	103.24
RBC *10^{12}/l	X	7.9	7.4	8.5	8.8	8.6	9.0	9.7	9.4	9.4
	SD	1.73	1.25	0.89	1.03	1.1	0.69	0.68	0.66	0.83
HGB g/l	X	98.4	91.3	94.8	93	92.5	96.7	106	107	103.5
	SD	18.18	19.32	11	11.92	14.54	9.25	4.42	7.32	9.02
HCT %	X	22.6	23.8	25.7	23.1	22.7	24	24.7	25	24.9
	SD	7.41	3.66	2.62	4.21	3.68	2.49	2.36	1.9	3.11

X - mean value; SD - standard deviation; significant differences between groups within a given sample are recorded using the same superscript: $^{\alpha, \beta}$ - $P < 0.05$; $^{a, b}$ - $P < 0.01$; $^{A, B}$ - $P < 0.001$; Group C - control group; Group A - experimental group 1 (α-tocopherol and Se parenteral application); Group B - experimental group 2 (β-carotene and α-tocopherol parenteral application); WBC - leukocytes; RBC - erythrocytes; HGB - haemoglobin; HCT - haematocrit; Lymph - lymphocytes; Mon - monocytes; Gran - granulocytes; PLT- platelets

Table 4. Concentration of selected biochemical and mineral indices in the calves' serum of at individual samplings.

Indicator		7–10 days of age			35 days after birth			70 days after birth		
		Group C	Group A	Group B	Group C	Group A	Group B	Group C	Group A	Group B
Protein g/l	X	62.12	60.29$^{\alpha}$	67.14$^{\alpha}$	58.03	58.95	57.30	60.28	62.64^{a}	58.33^{a}
	SD	7.45	6.60	4.31	3.96	4.33	3.40	2.66	3.57	3.18
Albumin g/l	X	30.88	31.68	30.50	32.40	33.24	32.96	34.67	34.94$^{\alpha}$	33.39$^{\alpha}$
	SD	1.51	0.95	1.91	2.05	1.89	2.12	1.52	0.93	1.74
Globulin g/l	X	31.24	28.61^{a}	36.64^{a}	25.63	25.71	24.34	24.69	27.70$^{\alpha}$	24.94$^{\alpha}$
	SD	7.24	6.79	3.52	3.38	3.74	2.53	3.54	3.78	2.57
A/g	X	1.06	1.17	0.84	1.28	1.32	1.37	1.41	1.28	1.35
	SD	0.35	0.29	0.09	0.20	0.23	0.16	0.43	0.20	0.15
Bilirubin µmol/l	X	6.09^{A,a}	3.34$^{A,\alpha}$	4.59$^{\alpha,a}$	3.19	4.52	4.40	4.92	4.00	4.67
	SD	1.94	0.95	0.73	1.62	1.29	1.58	1.89	1.05	1.39
AST µkat/l	X	0.69	0.62	0.70	0.77	0.82	0.78	1.63^{a,b}	1.25^{a}	1.20^{b}
	SD	0.29	0.14	0.17	0.18	0.12	0.14	0.30	0.28	0.36
GMT µkat/l	X	3.67	3.64	4.70	0.85	0.69	0.88	0.32^{a}	0.34$^{\alpha}$	0.42$^{a,\alpha}$
	SD	1.93	2.44	1.94	0.56	0.34	0.28	0.05	0.05	0.09
Urea mmol/l	X	2.54	2.09$^{\alpha}$	2.87$^{\alpha}$	2.87^{a}	2.40^{a,b}	2.28^{b}	2.20	2.41	2.25
	SD	0.87	0.60	0.54	0.46	0.38	0.40	0.09	0.18	0.13
Creatinine µmol/l	X	104.61^{a}	88.95^{a}	102.90	87.21$^{\alpha,a}$	98.23$^{\alpha}$	103.03^{a}	105.70	102.70$^{\alpha}$	111.57$^{\alpha}$
	SD	15.66	6.67	11.47	6.81	13.74	7.29	9.07	8.98	6.56
CK µkat/l	X	1.91	1.75	1.65	2.42	2.26	2.40	4.55	4.21	4.85
	SD	0.62	0.39	0.37	0.73	0.85	0.52	1.00	1.27	2.40
Haptoglobin g/l	X	0.28	0.26	0.24	0.31$^{\alpha}$	0.29	0.24$^{\alpha}$	0.31	0.29	0.28
	SD	0.05	0.05	0.08	0.06	0.06	0.07	0.07	0.09	0.09
Mg mmol/l	X	1.02	1.03	1.06	0.92^{A}	0.93^{B}	0.83^{A,B}	1.03	1.04	1.08
	SD	0.09	0.08	0.07	0.05	0.04	0.07	0.09	0.09	0.05
Fe µmol/l	X	13.91	15.89	15.96	19.54	22.00	18.96	27.60	25.10	19.84
	SD	14.15	10.20	8.86	15.45	16.55	11.12	5.89	7.34	9.46

X - mean value; SD - standard deviation; significant differences between groups within a given sample are recorded using the same superscript: $^{\alpha,\beta}$ - $P < 0.05$; ^{a,b} - $P < 0.01$; ^{A,B} - $P < 0.001$; Group C - control group; Group A - experimental group 1 (α-tocopherol and Se parenteral application); Group B - experimental group 2 (β-carotene and α-tocopherol parenteral application); A/g - albumin/globulin ratio; AST - aspartate aminotransferase; GMT - gamma-glutamyl transferase; CK - creatine kinase; Mg - magnesium; Fe - iron

Discussion

In this study, β-carotene supplementation had no effect on serum retinol concentrations in calves. Retinol itself, as well as β-carotene, can be stored in other tissues, mainly in fat and liver, for later use by the organism (Bryant et al. 2010). Some studies have confirmed that the retinol concentration in plasma reflects the actual state of retinol in case its stores in the liver are depleted (Ross et al. 2000; Bryant et al. 2010). Its direct supplementation (parenteral/oral) has been shown to be effective in increasing the retinol concentration in blood (Wise et al. 1946; Puvogel et al. 2008). On the other hand, according to some studies, supplementation with β-carotene as a retinol precursor was no longer as effective in terms of retinol concentration in the blood of calves, or had only minor effects on its concentration (Otomaru et al. 2018). Similarly, in our recent work Kadek et al. (2021a), β-carotene administered parenterally to highly pregnant cows had no significant effect

on retinol concentrations in calves. However, β-carotene concentrations in these calves were significantly higher compared to calves, whose mothers were not supplemented with β-carotene. Similarly, in our other study from the same year, parenteral supplementation of cows with β-carotene had no effect on their serum retinol concentrations (Kadek et al. 2021b).

Several studies have confirmed that it is possible to increase or maintain sufficient concentrations of β-carotene or α-tocopherol in the blood/serum of cattle by parenteral application (Sobiech et al. 2015; Otomaru et al. 2018; Hye et al. 2020). In the present work, we observed similar results in the parenteral application of a preparation containing β-carotene directly to calves. The concentration of β-carotene was the highest in the calves of group B at the second sampling. This indicates that the application of the preparation containing β-carotene had a significant effect on its concentrations in the calves' serum. In our aforementioned work (Kadek et al. 2021a), we observed interesting results when β-carotene was administered parenterally to pregnant cows 10–14 days before parturition. Calves from supplemented cows had a higher β-carotene concentration even before receiving the first dose of colostrum. In the current work, β-carotene concentration decreased at the third sampling together with α-tocopherol concentrations. Similarly, the highest concentrations of α-tocopherol in calf serum were reached by calves in group B at the second sampling. Despite its decline at the third sampling, group B maintained numerically the highest concentration of α-tocopherol at both post-application samplings. Calves face increased oxidative stress during weaning and therefore, have lower concentrations of vitamins and antioxidants in their plasma/serum (Lashkari et al. 2022). In the aforementioned study by Lashkari et al. (2022), the addition of 200 mg/kg RRR-alpha-tocopheryl acetate was the only effective dose of vitamin E to maintain plasma vitamin E concentrations in post-weaning calves at levels similar to pre-weaning concentrations.

The decrease in α-tocopherol and β-carotene concentrations in the third sample, i.e. at 70 days of age, was probably related to weaning and all the factors associated with it. Weaning is an important and demanding period in a calf's life. In the study of Bordignon et al. (2019), parenteral administration of a preparation containing minerals (Cu, Zn, Mn, Se) and vitamins (A and E) increased growth performance and strengthened the immune and antioxidant systems of calves in the nutritional transition period during the summer. Calves treated with this preparation had a higher amount of neutrophils and monocytes. During weaning, calves can undergo major changes, especially in connection with transportation to another place. In addition, the transition from individual shed housing to shared housing presents them with social stress as well. Stress can weaken the calf's immunity and challenge its antioxidant system (Inanami et al. 1999; Mattioli et al. 2020).

On the other hand, the α-tocopherol concentration in RBC was not significant between groups within individual samples. Similar results were observed in our previous work (Kadek et al. 2022). In the mentioned work, the α-tocopherol concentrations in plasma and RBC in individual categories of adult cows and calves at one month of age were compared. In contrast to plasma, where α-tocopherol concentrations were more variable between cow's categories, differences in α-tocopherol concentrations in RBC between the individual categories of cows were non-significant. In the work of Weiss et al. (1992), the ratio of α-tocopherol in RBC to plasma was not affected by vitamin E supplementation. On the other hand, it was variable in terms of pregnancy and lactation. The work of Roquet et al. (1992) suggests that the vitamin E concentrations in erythrocytes could be a better indicator of the overall status of vitamin E than its plasma concentration.

Regarding the Se concentration in the blood of calves in this study, the parenteral application of a preparation containing α-tocopherol and Se proved to be effective at the third sampling, i.e. after two injection applications. In contrast to α-tocopherol and

β-carotene, the Se concentration increased and reached the highest values in calves of group A (supplemented with α-tocopherol and Se) within the weaning period. An increase in the GPx activity was also observed in this group, as well as its highest values within the last, third sampling. It is well known that GPx is a selenoenzyme and its activity is dependent on Se. It plays an important role in scavenging free radicals. Se supplementation can increase GPx activity, and GPx activity was found to be higher in calves that were supplemented with oral triglycerides of selenate compared to the control group (Żarczyńska et al. 2021)

MDA is used as a biomarker for lipid peroxidation and oxidative damage (see the review by Niki 2014). Lipids are among the biological molecules most susceptible to damage by reactive oxygen species and reactive nitrogen species. Therefore, lipoperoxidation products such as MDA are the focus of interest as biomarkers of oxidative stress (Khoschsorur et al. 2000). Cows with diagnosed puerperal metritis had increased concentrations of MDA in the blood and at the same time significantly reduced concentrations of retinol and α-tocopherol compared to the control group. Numerically reduced but non-significant concentration of β-carotene was also observed (Mikulková et al. 2020). In the current study, the MDA concentration between groups of calves varied during sampling. No significant differences in MDA concentrations were observed between the groups at the first sampling. At the second sampling, the lowest MDA concentration was observed in group B. In contrast, at the third sampling it was observed in the control group. However, the overall numerically lower values of MDA were maintained in group B in both samples after the application of the vitamin.

The control group showed the highest values of TAC 30 days after the application of vitamins. The situation changed during the third sampling (75 days after the vitamin application), when group A showed the highest values of TAC. In the above mentioned study (Mikulková et al. 2020), no significant difference in TAC concentrations was found between healthy cows and cows with metritis. This was despite the fact that cows diagnosed with metritis had lower vitamin concentrations than cows in the control group in the study. Calves of mothers injected with a preparation containing α-tocopherol, retinol and β-carotene at a high stage of pregnancy showed an increased TAC in the serum (Kadek et al. 2021a). The TAC concentration expresses the antioxidant defence capacity of all antioxidant mechanisms and thus provides information on the balance of pro-oxidants and antioxidants (Ghiselli et al. 2000). Therefore, it also includes GPx activity and Se concentration as measured in our study, as well as all other antioxidants present in the body. GPx and Se were found at the highest levels in group A (which received parenterally administered α-tocopherol and Se) at the third sampling. It is well-known that Se and vitamin E act synergistically in the antioxidant system.

However, there are some studies that show the influence of vitamin or Se supplementation on red line haematological indices (Moosavian et al. 2010; Snarska et al. 2018) or on the count of total/individual WBC count (Otomaru et al. 2015; Bordignon et al. 2019). In this work, we did not find any significant difference in haematological parameters that would indicate the effect of selected vitamin formulas. Vitamin E protects the bilateral membrane of RBC from lipoperoxidation and is therefore necessary for the stabilization of erythrocytes in calves. In the study of Siddons and Mills (1981), vitamin E supplementation did not affect haematological parameters in calves. On the other hand, increased auto/peroxidative red blood cell haemolysis was observed in calves with low vitamin E intake. In the work of Bordignon et al. (2019), no significant differences in erythrocyte count, haemoglobin concentration, and haematocrit were observed between groups over time. However, a higher amount of leukocytes, neutrophils and monocytes was observed in the treated group at day 45 post-weaning.

In this study, the determination of biochemical and other mineral indicators (Fe and Mg) served to control the overall health and metabolism of the calves. As for the biochemical

indicators, lower values of protein, albumin, and urea mainly within the first and second sampling indicate a lower intake of nitrogenous substances (Bull et al 1991). Is generally known that increased creatine kinase (in this case, on day 70 of the calves' life) and at the same time, higher AST activity may indicate increased muscle breakdown, given that during this period the calves were exposed to increased stress situations (relocation to shared housing, dehorning). Magnesium concentrations were within reference values in the calves in this study. Iron concentration values were within the lower limits or deficient especially during milk nutrition (1st and 2nd sampling) (Bouda et Jagoš 1984). Iron deficiency in calves is usually associated in calves fed with whole milk (Matrone et al. 1957).

In conclusion, the repeated administration of vitamin/vitamin-mineral preparations to calves at 7–10 days of age and again at 35 days of age has an effect on calf serum vitamin concentrations and TAC. On the other hand, during periods of increased oxidative stress (weaning, transfer to shared housing and other aspects), the serum concentration of vitamins dropped sharply despite repeated parenteral vitamin/Se supplementation. Increased activity of GPx, Se, and TAC concentration were observed in the serum of calves receiving parenteral Se and α-tocopherol. Parenteral administration of α-tocopherol to calves had no effect on its concentration in calf RBCs. However, it decreased in all groups when moved to a shared housing, probably due to increased oxidative stress. Overall, it was shown that the group of calves that received a parenteral preparation containing α-tocopherol and β-carotene showed a higher concentration of β-carotene and lower MDA concentration, and thus a lower level of lipoperoxidation.

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