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Effect of parenteral supplementation of selenium and vitamin E on selected blood biochemical parameters in H-F cows during the transition period

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Summary

The aim of the study was to evaluate the influence of vitamin E and selenium supplementation on selected blood biochemical parameters in dairy cows during the transition period. The study was conducted on 20 Holstein-Friesian (HF) cows divided into two groups. Group I (experimental, 10 cows) was intramuscularly administered a vitamin E and selenium supplement (30 ml) (tocopherol acetate – 50 mg, sodium selenite – 0.5 mg, solvent – 1 ml) 5 days prepartum. Group II was the control with no supplementation. The BCS of all cows was determined at 4-4.2 five days prepartum. Blood samples were collected from all cows on 5 sampling dates (5 days prepartum, on the day of parturition day, and 5, 10 and 15 days postpartum). Serum total protein, glucose, cholesterol, triglyceride, NEFA (non-esterified fatty acid), BHB (beta-hydroxybutyrate), vitamin E and selenium levels were determined in the collected samples. AST (aspartate aminotransferase), GGTP (gamma-glutamyl transpeptidase) and GSH-Px (glutathione peroxidase) activity was measured. In the experimental group, a significant increase in selenium and vitamin E concentrations was observed on the day of parturition, and an increase in GSH-Px activity was noted 5 days postpartum. No significant changes in the monitored parameters were reported in the control group.

Keywords: dairy cows, transition period, selenium, vitamin E

The transition period between 3 weeks prepartum to 3 weeks postpartum is the most sensitive period during the reproductive cycle of dairy cows. The most commonly noted perinatal disorders in dairy cows include clinical mastitis (16.5% of animals), hoof infections (14.0%), placenta retention (7.8%), recumbency (4.9%), uterine infections (4.6%) and left displacement of the abomasum (3.5%) (34). Clinical ketosis affects 7-15% of animals, depending on the type of herd and the applied diagnostic method. Subclinical ketosis is reported in 15-43% cows. Most animals (more than 50%) are affected by more than one disorder (27), and fatty liver disease also poses a significant problem in the perinatal period.

Selenium plays a very important role in higher organisms. This essential trace element inhibits glycolysis, protects the body against the toxic effects of cadmium, lead and mercury salts, ionizing radiation and nitrosamines (21). Selenium boosts humoral and cellular immunity. Cows experience metabolic changes during the transition period, including excessive production of free radicals that contributes to oxidative stress and directly impairs immune function. Research has demonstrated that selenium supplements administered to cows during the transition period significantly inhibit the production of free radicals and stimulate neutrophil activity. Selenium contributes to the maintenance of vascular homeostasis under exposure to

pro-oxidative stressors. There is a general scarcity of research documenting antioxidants' ability to reduce the risk of perinatal diseases in high-yielding cows. Hidiroglou and Hartin (17) did not observe any correlations between selenium supplementation and the prevalence of fatty liver disease. Another author (2) demonstrated *in vitro* that an increase in the concentrations of free fatty acids disrupts mitochondrial function by inhibiting oxidative phosphorylation and contributing to the production of free radicals, including superoxide radicals. Some authors (3, 39) observed that cows were more exposed to oxidative stress during the perinatal period; in particular animals that had a higher BCS and lost more weight postpartum. Vitamin E plays an equally vital role in living organisms. According to some authors (30, 31), vitamin E is probably the most important antioxidant in cell membranes where it protects polyunsaturated fatty acids against oxidation. In mammalian cells, α-tocopherol is present mainly in mitochondrial fractions and the endoplasmic reticulum (6). α-tocopherol exerts a protective effect by interacting with free radicals generated from polyunsaturated fatty acids (mainly in cell membranes) and producing stable lipid hydroperoxidases. Vitamin E boosts the immune system by activating chemical processes required for phagocytosis and lowering prostaglandin production in immune cells.

The data found in literature suggests that excessive postpartum fat mobilization in dairy cows disrupts metabolic processes, mostly glucose and fatty acid metabolism, and that antioxidants can reduce liver loading and stimulate liver metabolism. The objective of this study was to determine the effect of parenteral administration of a selenium and vitamin E supplement on energy and fat metabolism in high-yielding dairy cows in the transition period.

Material and methods

The experiment was conducted on 20 pregnant Holstein-Friesian (HF) cows in their third lactation, fed TMR and reared in a free-stall system in the same barn situated in Warmia and Mazury, a Polish region that is generally regarded as most deficient in selenium. Towards the end of the dry period, the animals were fed a TMR in the daily amount of 22 kg, comprising grass silage (12.5%), maize silage (63%), wheat pellets (9%), straw (*ad libitum*), soybeans (14.5%) and a mineral supplement for dry cows (1%). After parturition, cows were fed a TMR in the daily amount of 42 kg, comprising grass silage (14.5%), maize silage (41%), wheat pellets (18%), straw (*ad libitum*), rapeseed oil (9.8%), beet pulp (8%), soybeans (7.7%) and a mineral supplement for dairy cows (1%).

The milk yield during the previous 305-day lactation period was evenly distributed at approximately 8300--8600 kg. Cows with BCS of 4.0-4.2 towards the end of the dry period were selected for the study.

The cows were divided into two groups of 10 animals each. Group I (experimental) was intramuscularly administered a vitamin E and selenium supplement (30 ml) (tocopherol acetate – 50 mg, sodium selenite – 0.5 mg, solvent – 1 ml) 5 days prepartum. Group II was the control with no supplementation.

Blood was sampled from the caudal vein into test tubes containing a coagulation activator in the morning hours on the following sampling dates: 5 days prepartum (5DPP), on the day of parturition (DP), 5 days postpartum (5DPoP), 10 days postpartum (10DPoP) and 15 days postpartum (15DPoP). The samples were centrifuged at 2000 G for 10 minutes to separate the serum. Serum glucose, total protein, triglyceride and cholesterol concentrations were determined using the Cormay Accent 200 biochemistry analyzer. The concentrations of not esterified fatty acids were determined by the Wako NEFA-HR assay (ACS-ACOD method), and beta-hydroxybutyrate levels (BHB) were determined with the use of the Wako Autokit 3-HB. Serum activity of aspartate aminotransferase (AST) and gamma-glutamyl transpeptidase (GGTP) was determined using the Cormay Accent 2000 biochemistry analyzer.

The activity of selenium-dependent glutathione peroxidase (GSH-Px) was determined in whole blood by the kinetic method with cumene hydroperoxide and phosphate buffer (Ransel kit).

Selenium concentrations were determined in serum samples (1 ml) mineralized in a 3 : 1 mixture of nitric acid and perchloric acid. The samples were mineralized in an electric aluminum block heating digester with temperature control for 2-3 hours at 120-200°C. The obtained colorless solution was cooled, combined with concentrated hydrochloric acid and heated at 80°C for 20 minutes to reduce Se^{VI} to Se^{IV}. Reagent samples and samples containing Se (0.050 and 0.100 μg/g serum) were prepared simultaneously, and Se recovery was 98.3% and 99.4%, respectively. The applied analytical method was validated by analyzing certified reference material BCR No. 184 Lyophilized Bovine Muscle, certified value – 0.183 μg/g, determined value – 0.171 μg/g. The selenium content of mineralized samples was determined by hydride generation flame atomic absorption spectrometer (air-acetylene flame) in a Solar Unicam 939 spectrometer equipped with an Optimus data station, a deuterium lamp for background correction and the Unicam VP 90 hydride generator. Serum levels of vitamin E were determined by high-performance liquid chromatography in the Hewlett Packard HP-1050 HPLC system with the use of Recipe Chemical ClinRep kits, at the flow rate of 1.5 ml/min and 325 nm wavelength.

The following serum selenium concentrations or blood GSH-Px activities were used as reference values (30) for the assessment of the selenium status: < 70 μg/l, or < 40 U/g Hb – deficient, 70 to 100 μg/l, or 30 to 70 U/gHb – marginal, > 100 μg/l, or > 70 U/gHb – adequate.

The results were analyzed statistically in onefactor orthogonal design. The significance of differences between mean values in groups and examinations were verified by the Student's t-test for $p \leq 0.01$ using Statistica 10.0 software.

Results and discussion

In the Table 1 was given.

Total protein concentrations in serum were similar in experimental and control group animals and remained within the reference range in all tests. A minor increase in total protein levels was noted postpartum, but the observed changes were not statistically significant. A reverse trend was reported in glucose concentrations. The lowest glucose levels were reported 10 days postpartum, and the highest values were noted prepartum, but no significant differences were observed between sampling dates or animal groups. Cholesterol concentrations peaked on the last sampling date (15 days postpartum) in both experimental and control group animals. No significant differences in cholesterol levels were noted between groups. Interestingly, cholesterol concentrations were relatively low on the day of parturition. Triglyceride levels were similar in both groups, and a minor (not statistically significant) increase in this parameter was reported on the day of parturition in the control group. Non-esterified fatty acids (NEFA) concentrations in both groups increased on successive sampling dates, but remained within the reference range, and the observed differences were not statistically significant. Beta-hydroxybutyrate (BHB) concentrations were a little bit higher in control group animals on all sampling dates (the greatest difference was reported 5 days postpartum), but the noted differences were not statistically significant.

In the Table 2 was given.

Selenium and vitamin E concentrations were significantly higher in experimental group animals, and the increase of vitamin E was manifested earlier with regard to Se levels. Se concentrations increased significantly in experimental cows beginning on the second sampling date and remained at a high and stable level until the end of the experiment. Vitamin E concentrations increased significantly in the experimental group beginning on day 5 postpartum (third sampling date). The activity of selenium-dependent glutathione peroxidase (GSH-Px) increased significantly in the

Tab. 1. Serum total protein, glucose, cholesterol, triglyceride, non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB) concentrations in experimental and control group cows

Days	TP g/l		GLU mmol/l		CHOL mmol/l		TG mmol/l		NEFA mmol/l		BHB mmol/l	
	Se GR	No Se GR	Se GR	No Se GR	Se GR	No Se GR	Se GR	No Se GR	Se GR	No Se GR	Se GR	No Se GR
5 DPP	66.22	65.08	4.08	4.50	2.92	2.60	0.28	0.20	0.37	0.31	0.38	0.42
	± 9.22	± 6.23	± 1.01	± 0.59	± 0.74	± 0.62	± 0.15	± 0.18	± 0.04	± 0.09	± 0.09	± 0.11
DP	68.02	65.67	3.23	4.27	2.65	2.09	0.32	0.23	0.41	0.38	0.42	0.52
	± 6.58	± 8.65	± 0.94	± 1.12	± 0.65	± 0.87	± 0.22	± 0.10	± 0.12	± 0.12	± 0.10	± 0.18
5 DPoP	69.80	67.92	3.05	3.24	2.85	2.55	0.23	0.19	0.55	0.63	0.31	0.51
	± 6.69	± 8.23	± 0.78	± 0.93	± 0.41	± 0.45	± 0.19	± 0.07	± 0.13	± 0.15	± 0.12	± 0.22
10 DPoP	71.54	69.42	3.06	3.09	4.05	3.52	0.26	0.21	0.71	0.82	0.36	0.45
	± 7.28	± 5.87	± 0.91	± 0.72	± 0.58	± 0.51	± 0.07	± 0.09	± 0.21	± 0.23	± 0.17	± 0.12
15 DPoP	72.38	75.34	3.23	3.56	4.83	4.51	0.28	0.24	0.78	0.86	0.33	0.37
	± 9.26	± 6.48	± 0.86	± 0.98	± 0.56	± 0.48	± 0.12	± 0.11	± 0.18	± 0.17	± 0.11	± 0.21

Tab. 2. Selenium and vitamin E concentrations, and activity of glutathione peroxidase, aspartate aminotransferase (AST) and gamma-glutamyltranspeptidase (GGTP) in experimental and control group cows

Days	Se µg/l		Vit E µg/ml		GSH-Px U/gHb		AST U/l		GGTP U/l	
	Se GR	No Se GR	Se GR	No Se GR	Se GR	No Se GR	Se GR	No Se GR	Se GR	No Se GR
5 DPP	69.24	66.36	3.74	3.24	22.81	19.00	74.00	94.00	21.80	24.60
	± 9.36	± 7.11	± 1.03	± 0.89	± 7.67	± 7.10	± 15.86	± 14.96	± 5.65	± 4.98
DP	98.43^{xa}	62.43	4.36	3.80	31.25	19.08	102.00	105.00	24.66	25.67
	± 12.24	± 5.98	± 0.93	± 0.77	± 10.45	± 8.09	± 32.59	± 23.24	± 7.56	± 7.35
5 DPoP	94.67^{xa}	63.87	6.41^{xa}	4.10	69.56^{xa}	19.51	103.75	102.40	19.00	22.80
	± 10.71	± 8.32	± 0.89	± 1.15	± 37.36	± 5.32	± 29.46	± 29.14	± 4.21	± 9.15
10 DPoP	90.51^{xa}	55.22	5.77^{xa}	4.32	67.18^{xa}	25.90	86.60	100.90	20.60	24.90
	± 6.94	± 10.65	± 1.07	± 1.04	± 34.99	± 10.11	± 20.48	± 18.36	± 7.48	± 10.02
15 DPoP	91.00^{xa}	55.83	5.23^{xa}	4.28	77.28^{xa}	22.23	89.00	97.20	22.40	23.80
	± 7.56	± 8.59	± 1.12	± 1.10	± 37.82	± 9.46	± 21.43	± 14.87	± 7.01	± 5.92

Explanations: x – statistically significant difference between groups; a – statistically significant difference between sampling dates

experimental group, and the highest GSH-Px levels were observed on the last day of the experiment. On the last three sampling dates, GSH-Px activity was significantly higher in experimental than in control group animals. A high correlation between Se and GSH-Px was observed (Fig. 1). The lowest levels of aspartate aminotransferase (AST) activity were observed on the first sampling date in both groups. AST activity increased insignificantly on two successive sampling dates and decreased toward the end of the experiment, but the noted differences were not statistically significant. Minor fluctuations in gamma-glutamyl transpeptidase (GGTP) activity were noted in both groups, but the observed differences were not statistically significant.

Total serum protein concentrations in experimental and control group animals increased insignificantly during the experiment, but remained within the reference range. During the transition period, dairy cows undergo metabolic adaptations to support milk production and maintenance of lactation, and those processes involve fat, protein and mineral mobilization (11). The mobilization of skeletal muscle proteins is intensified during early lactation to compensate for the energy deficit (23, 28). The amount of energy that can be mobilized from protein in response to a negative energy balance is limited in comparison with the energy mobilized from fat reserves (12). Research has demonstrated that protein mobilization in cows ends around 4 weeks postpartum, whereas fat mobilization continues for at least 8 weeks postpartum (22). In this study, Se and vitamin E supplementation had no significant influence on serum protein concentrations in experimental group cows. Helal et al. (16) observed an increase in serum total protein levels in cows fed a selenium-supplemented diet in the last stage of pregnancy in comparison with animals not receiving the supplement during the same period. El-Shahat and Abdel Monem (13) administered selenium and vitamin E supplements to ewes two weeks before mating and during pregnancy, and reported a significant increase in total protein concentrations in comparison with control group ewes whose feed was not supplemented.

An insignificant decrease in serum glucose levels was observed in experimental and control group animals in the first and second week of lactation. In response to a negative energy balance, intensified adipose tissue lipolysis, which can occur already in the last week of pregnancy, weakens gluconeogenesis and contributes to hypoglycemia in cows during early lactation (20). A temporary drop in glucose levels in the first weeks of lactation due to higher lactose synthesis and decreased gluconeogenesis was also observed by Doepel et al. (10), and it is consistent with our findings. Nayyar et al. (24) reported significantly higher blood glucose levels in heifers receiving selenium and vitamin E supplements in comparison with control

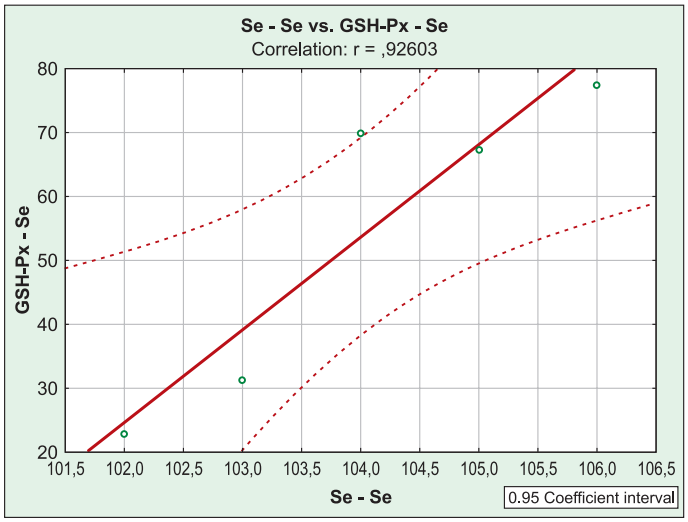

Fig. 1. Correlation coefficient between GSH-Px and Se

group animals. The results reported by Stapleton (40) suggest that selenium supplementation *in vivo* and *in vitro* participates in insulin-like activities, including stimulation of glucose uptake and regulation of enzymes involved in glycolysis, gluconeogenesis, fatty acid synthesis and the pentose phosphate pathway. In our study, selenium supplementation had no significant effect on blood glucose levels in experimental cows, which could indicate that the above processes were not triggered in response to the applied selenium dose.

Insignificantly higher serum cholesterol concentrations were noted in experimental cows that were parenteral supplemented with selenium and vitamin E. Similar results were reported by Nayyar et al. (25) who administered selenium and vitamin E to cows 14 and 7 days prepartum. Weiss et al. (42) did not observe any correlations between vitamin E supplementation and serum cholesterol levels in dairy cows during the transition period. In cows, variations in serum cholesterol concentrations are associated with changes in lipoprotein synthesis in hepatocytes. An increase in the circulating levels of high-density lipoproteins (HDL), a rapid decrease in the levels of low-density lipoproteins (LDL) and a decrease in the levels of very-low-density lipoproteins (VLDL) are observed postpartum. LDL are characterized by the highest share of cholesterol and act as the main carriers of cholesterol from the liver to other organs (33, 38). In the current study, the discussed mechanisms lowered serum cholesterol concentrations in high-yielding cows on the day of parturition and in the first days postpartum.

No significant differences in serum triglyceride levels were observed between experimental and control group animals. In dairy cows, which are characterized by high free amino acid concentrations in the blood, lipid mobilization begins in late pregnancy and reaches its peak during early lactation. Released fatty acids are re-esterified and accumulated as triglycerides in the liver, mainly due to the decreased ability of hepa-

tocytes to transport lipids via VLDL (9). Njeru et al. (26) did not observe significant correlations between dietary inclusion levels of vitamin E, and triglyceride and cholesterol levels in sheep and cows, which is consistent with the results of our study. According to Falkowska et al. (14), vitamin E and selenium can modify lipid metabolism in cows during early lactation by increasing triglyceride and HDL levels.

Non-esterified fatty acid (NEFA) concentrations in the blood serum of experimental and control group cows increased during the experiment. Dann et al. (8) observed an increase in NEFA concentrations postpartum. The magnitude of that increase was inversely proportional to the intake of feed dry matter and was highly correlated with the degree of adipose tissue lipolysis (19). Guo et al. (15) demonstrated that serum NEFA levels are a robust indicator of lipid mobilization and that they increase significantly during rapid weight loss after parturition, which is correlated with the risk of fatty liver disease. In our study, excessive fat mobilization was not reported in the analyzed animals, and selenium and vitamin E supplementation had no significant influence on NEFA concentrations. The latter can probably be attributed to the low intensity of adipose tissue lipolysis in experimental and control group cows and, consequently, low oxidative stress in response to free radical generation.

Beta-hydroxybutyrate (BHB) concentrations were low in both animal groups on all sampling dates. Insignificantly higher BHB levels were noted in control cows. BHB is a ketone body that is used to diagnose subclinical and clinical ketosis. According to Saun (34), serum BHB concentrations in postpartum cows exceed 1.1 mmol/l in subclinical ketosis and 2.4 mmol/l in clinical ketosis. Ketosis was not observed in our study, although the animals selected for the experiment had relatively high BCS (4.0-4.2), which could suggest a higher propensity to develop this condition. Contradictory findings were reported by Studer (41), who observed moderate postpartum ketosis in nearly all cows with prepartum BCS higher than 3.5. In our study, selenium and vitamin supplementation did not affect BHB concentrations. In the work of Calamari et al. (5), the administration of selenium to heat-stressed transition cows lowered BHB levels, but the mechanism of action responsible for the above changes remains unknown. It can be assumed that the metabolic response (including the synthesis of ketone bodies) to heat stress and oxidative stress during the transition period may be modulated by selenium's antioxidant properties.

Selenium concentrations in experimental group animals were significantly higher on four successive sampling dates in comparison with control animals. The highest selenium concentrations were noted on the day of parturition, i.e. 5 days after parenteral supplementation. The observed rate of changes in selenium concentrations was similar to that reported by Slavik et al. (36) who noted the highest serum selenium levels two days after parenteral administration of the analyzed element. In a study of calves (43), selenium was less readily absorbed when administered intramuscularly. The form and manner of administration are critical success factors in selenium supplementation. Qin et al. (32) demonstrated that orally administered selenium yeast was significantly more effective than inorganic selenium because yeast contains organic selenium, mostly selenomethionine, the most absorbable form of this element. Inorganic selenium compounds contain selenates that are easily reduced by ruminal microflora to insoluble and non-absorbable elemental selenium (7). In this study, serum selenium concentrations reached 98 μg/l and remained fairly stable until the end of the experiment, indicating that the applied dose of the intramuscularly administered selenium effectively covered the demand for this element. In the control group, selenium concentrations were significantly lower, reaching values which, according to Pavlata et al. (9), are indicative of a significant selenium deficiency.

Serum concentrations of vitamin E in experimental cows increased on the third sampling date and were elevated until the end of the experiment in comparison with control. A gradual increase in vitamin E levels was observed in control group animals throughout the study. The increase in vitamin E concentrations noted in experimental animals 5 days postpartum was directly correlated with the administration of supplements containing selenium and α-tocopherol. Other authors (18) demonstrated that parenteral administered natural forms of tocopherol (D-α-tocopherol) were more readily available than synthetic tocopherol (DL-α--tocopherol). Alcohol-based formulations of vitamin E are characterized by greater therapeutic efficacy than ester compounds such as acetates. In our study, a commercial preparation containing tocopherol acetate was used on account of its popularity and market availability. The observed rate of changes in α-tocopherol concentrations, which peaked 10 days after supplementation, was similar to that reported by other authors (4, 37). It should be noted, however, that vitamin E levels decreased on successive sampling dates, which indicates that a single dose delivers relatively short-lived effects. For maximum efficacy, vitamin E supplements should be administered in several doses, for example at weekly intervals, but without the addition of potentially toxic selenium. Lower serum vitamin E concentrations were reported in control group animals prepartum. Our results are consistent with the findings of Weiss et al. (42) who observed a significant drop in serum vitamin E levels in dry cows in the last trimester of pregnancy. In our experiment, the postpartum increase in vitamin E concentrations in control group animals can probably be attributed to changes in diet (nutrient-

dense TMR and higher feed intake) that significantly increased the amount of orally ingested α-tocopherol.

Glutathione peroxidase (GSH-Px) activity in red blood cells increased significantly in experimental group cows beginning on day 5 postpartum and was positively correlated with selenium concentrations. According to other authors (29), GSH-Px activity is a robust indicator of selenium status. GSH-Px activity in red blood cells reflects the long-term selenium status, whereas serum selenium levels are indicative of rapid and short-lived changes in its concentrations. In experimental group cows, GSH-Px activity increased 10, 15 and 20 days after supplementation. Similar results have been reported by other authors (29) who observed an increase in GSH-Px activity in the same period. The time (approximately 2 weeks) between Se supplementation and the observed increase in GSH-Px activity is determined by the mechanism of Se deposition in red blood cells during erythropoiesis and by GSH-Px biosynthesis (Se is incorporated into the active site of the enzyme in the form of selenocysteine) (1). In control group cows, GSH-Px activity was significantly lower throughout the entire experiment. Bernabucci et al. (3) reported a significant drop in GSH-Px activity in cows between day 4 prepartum and day 11 postpartum. The authors attributed the observed changes to a significant deterioration in the oxidative status of transition cows. Our results seem to validate the above hypothesis and indicate that changes in fetal metabolism in the last stage of pregnancy and intensified metabolic processes in cows during early lactation can significantly impair systemic mechanisms that influence GSH-Px protection, as demonstrated by its low activity.

Aspartate aminotransferase (AST) activity was similar in both groups, and the lowest levels of AST activity were observed 5 days prepartum. Similar results were reported by Seifi et al. (35), in whose study a significant decrease in AST activity was noted in transition cows 20 days prepartum, followed by an increase until 21 days postpartum. High AST values are positively correlated with fatty liver disease and ketosis (41) and can be used to monitor those conditions. Our findings were not indicative of metabolic disorders. The activity profile of gamma-glutamyl transpeptidase (GGTP) was similar to that of AST. GGTP is present in cell membranes, and it is an effective diagnostic indicator of fatty liver disease, cirrhosis and biliary problems. Similarly to this study, Joksimović-Teodorović et al. (20) did not observe significant changes in GGTP activity in healthy transition cows. They also reported a significant correlation between higher levels of GGTP activity and the progression of fatty liver disease in dairy cows.

In the present study, no significant changes in the analyzed parameters of protein, carbohydrate and fat metabolism were observed in transition cows. Selenium and vitamin E supplements had no significant effect on the above mentioned indicators. A single dose of selenium and vitamin E administered 5 days prepartum fulfilled the demand for selenium in high-yielding cows during the transition period, but it was not sufficient to supply them in vitamin E, so it can be concluded that the dose of this vitamin should be repeated. The results of the study indicate, however, that supplementation with vitamin E and selenium preparations may have a positive impact on the health of the cows during the transition period due to the reduction in the risk of oxidative stress and its associated consequences.

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