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Original article

The influence of short-term selenitetriglycerides supplementation on blood selenium, and hepatic, renal, metabolic and hematological parameters in dairy cows

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Abstract

Selenium deficiency is a common nutritional disorder in dairy cattle globally. However, selenium supplementation can lead to selenium toxicity. This study evaluated a novel, low-toxicity selenium supplement, selenitetriglycerides, to determine its efficacy and safety in dairy cows. The study was conducted on 12 Holstein Friesian cows divided in two equal groups (control group without supplementation of selenium and experimental group with supplementation of selenitetriglycerides). Experimental cows (n=6) were orally administered 300 mg/cow/day of selenitetriglycerides for 14 days (days 1-14) and then monitored for a further 14 days (days 15-28). Blood from both groups of cows was sampled for determination of selenium concentrations, activity of aspartate aminotransferase, creatine kinase, lactate dehydrogenase, gamma-glutamyl transferase, concentrations of triglycerides, cholesterol, non-esterified fatty acids, glucose, total protein, urea, creatinine and hematological parameters. Serum selenium concentrations in the experimental group increased significantly on day 2 (from 64.92±6.89 µg/L to 127.95±13.75 µg/L), peaked on day 7 (266.22±14.21 µg/L) and remained significantly above the initial baseline values (day 1) for 28 days. Serum selenium concentrations in the control group did not change significantly during the 28 day period (65.22 µg/L on 1st day and 64,35 µg/L on 28th day) and were significantly lower than those in the experimental group from day 2 to day 28. The results of clinical examinations, analyses of hematological parameters, and liver and kidney function tests showed that selenitetriglycerides had no adverse effect on the health or on the metabolic or haematological statuses of the cows. These findings indicate that selenitetriglycerides are safe and effective selenium supplements for cattle.

Key words: selenium, selenitetriglycerides, cattle, biochemical parameters, haematology

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Introduction

Selenium deficiency can have serious health implications for animals, including nutritional muscular dystrophy in lambs and calves, pancreatic necrosis and exudative diathesis in birds, and reproductive problems in rams and bulls. Selenium deficiency can also compromise growth, development and survival in calves, and/or increase the animals' susceptibility to various, mostly infectious, diseases (Spears et. al 1986). Through incorporation into the active sites of glutathione peroxidase, thioredoxin reductase and iodothyronine deiodinase enzymes (Lu et al. 2009), selenium plays important roles in antioxidative processes, muscle tissue function and cancer prevention.

While selenium underpins important physiological functions, the difference between a healthy dose and a toxic dose is very small. Toxicity and efficacy are determined by several factors, including genetic predisposition, rate of elimination, form of the administered supplement, and interactions with feed ingredients (MacDonald et al. 1981). This study addresses the form of the selenium supplement.

Selenitetriglycerides are a novel group of semi-synthetic compounds which contain selenium and triglycerides and are derived by chemical modification of sunflower oil with selenic acid (Stańczyk et al. 2010). These compounds are synthesized by esterification of pre-oxidized triglycerides into hydroxyl derivatives with selenic acid. Selenitetriglycerides have lipophilic properties, and they are rapidly distributed in the body. In rats administered 2% and 5% selenitetriglycerides supplements, selenium concentrations were higher in the kidneys and the liver, and were lower in the testicles, brain, spleen, lungs, intestines and the heart. Jastrzebski et al. (1997) demonstrated that in rats, selenitetriglycerides are metabolized mainly in the liver and are excreted by the kidneys. In rats, the supplement was completely eliminated 24 hours after administration.

The minimal acute lethal dose of Se for livestock ranges from 1-5 mg/kg BW (Koller and Exon 1986). In rats, the average lethal oral dose of selenium (Se LD_{50}) was 100 mg/kg BW in 2% selenitetriglycerides supplements and 68 mg/kg BW in 10% supplements (Jastrzębski et al. 1997). These findings indicate that 2% selenitetriglycerides supplements are over 30-times less toxic that sodium selenite which produces lethal effects in rats at a dose of 3 mg/kg BW. Subcutaneous and parenteral administration of selenitetriglycerides at 500 and 100 mg Se (IV)/kg BW did not induce lethal effects or other clinical symptoms in rats.

To date there have been no published studies on the safety or efficacy of selenitetriglycerides supplements in cattle. The aim of this study was to determine the effects of short-term oral administration of a selenitetriglycerides supplement on serum selenium concentrations and selected hematological and biochemical parameters in dairy cows.

Materials and Methods

All experimental procedures were carried out in accordance with the recommendations of the Local Ethics Committee for Animal Experimentation.

Animals, diets and experimental design

The experiment was performed on 12 Holstein-Friesian cows on a dairy farm (~ 150 cows) in the north-eastern part of Poland. The cows were housed all year round in a freestall barn and fed a partial mixed ration (PMR) (Table 1) supplemented with a vitamin and mineral premix (Table 2) and concentrate at 6 kg/animal.

All animals were in their third lactation and of similar milk yield (mean+SD 305-d milk yield = 9.700±120 kg) and body weight (605±24 kg). The 12 selected cows were randomly assigned to one of two experimental treatment groups; experimental and control (six animals in each). Cows in the experimental group were supplemented with selenitetriglycerides (300 mg/cow/day) administered by an esophageal tube daily for 14 days from day 190 (day 1) to day 205 of lactation and were monitored for a further 14 days after supplementation. Cows in the control group were maintained on the same diet as that of the experimental group but did not receive selenitetriglycerides supplementation.

Clinical examination

Clinical examinations (rectal temperature, heart rate and respiratory rate, and a general health evaluation) were performed once daily by days 1, 4, 7, 14 and 28 of the experiment. In addition, all cows were continuously monitored by the breeder who was asked to report any changes in the animals' health, behavior (changes in appetite and thirst) and milk yield.

Sampling and analyses

Coccygeal blood samples were collected from both groups of cows in the morning (before the daily oral administration of selenitetriglycerides in the experimental group) into vacutainers containing K2 EDTA (Vacuette, Greiner Bio-One, France) for haematological analysis and into vacutainers containing a clot activator (Vacuette, Greiner Bio-One, France) for bio-

Table 1. Ration fed to the cows prior to and during the 28-day experiment.

Ingredient	kg/animal
Maize silage	23.9
Grass haylage	15.2
Rapeseed meal	1.3
Soybean meal, 46%	1.1
Vitamin and mineral premix	0.15
Agrocell (yeast)	0.05
Sodium bicarbonate	0.10
Limestone	0.10
Salt	0.05

Table 2. Composition of the vitamin and mineral premix fed to the cows prior to and during the 28-day experiment.

Ingredient	Quantity/kg
Calcium	150 g
Phosphorus	50 g
Magnesium	70 g
Sodium	60 g
Copper	2,500 mg
Zinc	7,000 mg (zinc chelate 3.900 mg)
Manganese	4,400 mg
Iron	2,700 mg
Iodine	145 mg
Cobalt	50 mg
Selenium	20 mg (organic Se 30 mg)
Vitamin A	900,000 IU
Vitamin D3	210,000 IU
Vitamin E	6,000 mg
Vitamin B1	100 mg
Vitamin B2	100 mg
Rumen-protected niacin	1,000 mg
Pantothenic acid	250 mg
Vitamin B6	50 mg
Folic acid	15 mg
Vitamin B12	3,200 µg
Biotin	100,000 µg

chemical analyses on days 1, 2, 3, 4, 5, 6, 7, 14, 18, 21 and 28.

Serum selenium concentration was determined by hydride generation-flame atomic absorption spectrometry (Unicam 939 Solar spectrophotometer). The following haematological parameters were determined in whole blood samples: white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet count (PLT), (Siemens ADVIA 2120 hematology analyzer, cytometric and laser reading). The following biochemical parameters were determined: activity of aspartate aminotransferase (AST), creatine kinase (CK), lactate dehydrogenase (LDH) and gamma-glutamyl transferase (GGT), and the concentrations of triglycerides (TG), cholesterol (CHOL), non-esterified fatty acids (NEFA), glucose (GLU), total protein (TP), urea (UREA) and creatinine (CREA), (Cormay ACCENT 200 automatic biochemical analyzer and Cormay diagnostic kits).

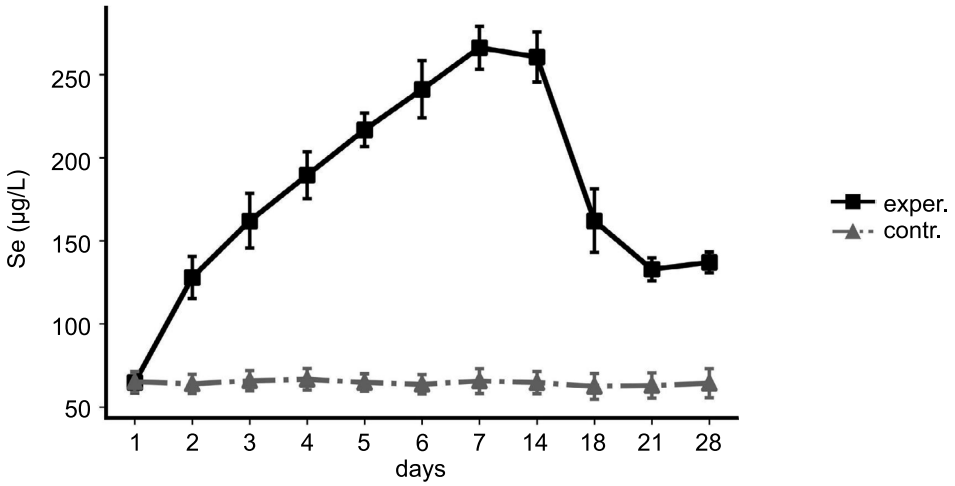

Fig. 1. Serum selenium concentration in supplemented and not supplemented cows (mean+SD)
Solid line – significant differences at P≤ 0.05 between sampling (day 1 vs. day 2; day 1 vs. day 3; day 1 vs. day 4; day 1 vs. day 5; day 1 vs. day 6; day 1 vs. day 7; day 1 vs. day 14; day 1 vs. day 18; day 1 vs. day 21; day 1 vs. day 28 in experimental and control groups) and between group (within days)

Statistical analysis

The results of laboratory tests were presented in SI units and subjected to statistical analysis. Data were entered into Microsoft Excel, and processed using SPSS software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). The significance of differences between sampling dates was determined for control and experimental groups using a p value of <0.05. The differences between the groups were determined by Mann Whitney U test. The differences between day 1 and the remaining sampling dates (days 2, 3, 4, 5, 6, 7, 14, 18, 21, 28) were calculated using the Wilcoxon signed-rank test with the Bonferroni adjustment for multiple comparisons.

Results

There were no significant changes in the clinical parameters over time or between the groups. Daily rectal temperature in the experimental and control group ranged from 38.2±0.4°C to 38.9±0.3ºC and 38.4±0.2 to 39.1±0.4, heart rate from 53.8±8.2/min to 61.3±10.2/min and 56±4.6/min to 62.9±8.7/min and respiratory rate from 26.7±5.3/min to 31.4±4.8/min and 25.5±6.6/min to 33.2±3.1/min, respectively. The breeder did not report any changes in the animals' behavior, appetite or milk yield or clinical signs indicative of disease during the four-week experiment.

Serum selenium concentrations in the experimental group were lowest on day 1 before the administration of the selenitetriglycerides supplement. On successive sampling dates, serum selenium concentration increased significantly until day 7, after which, it decreased until the end of the experiment. On day 28 (two weeks after the end of supplementation), serum selenium concentration was significantly higher than on day 1. Serum selenium concentrations in the control group did not change significantly during the experiment and were significantly lower compared to the experimental group from day 2 until the end of the experiment (Fig. 1). No significant changes in hematologic parameters were observed during the study between sampling dates or between groups of animals (Table 3). No significant differences in the mean activity of AST, LDH, GGT and CK were noted relative to the initial values or between the groups (Table 4). Only minor fluctuations in mean triglyceride concentrations were noted during the experiment in both groups of animals. No significant differences in mean serum cholesterol concentration were found between sampling dates or between groups of cows (Table 5). The highest average serum concentrations of NEFA in the experimental group were noted on day 1. During the experiment, NEFA values continued to decrease and were significantly lower on days 4 and 5 and days 21 and 28 relative to the first sampling date. Serum concentrations of NEFA in the control group were significantly higher than those in the experimental group during most of the experimental period – except the day 1 and 6 (Table 5). No significant differences in mean serum glucose concentration and total protein concentrations were observed during the experiment, and only minor fluctuations in the values of these parameters were noted in both groups (Table 5). No significant changes were observed in urea and creatinine concentrations during the entire experiment in both groups of animals (Table 5).

Table 3. Hematological parameters in cows with supplementation of selenitetriglycerides and control group (mean+SD).*

		Days										
Parameter		1	2	3	4	5	6	7	14	18	21	28
WBC (10^9/L)	Exper.	8.18 ±0.39	7.40 ±1.35	7.29 ±1.17	7.73 ±1.11	7.45 ±0.89	7.40 ±0.90	7.70 ±0.84	7.99 ±0.66	7.37 ±0.80	7.41 ±0.77	8.09 ±1.12
	Contr.	8.49 ±0.98	7.64 ±0.70	7.85 ±0.92	8.00 ±0.96	7.72 ±0.55	7.98 ±0.99	7.99 ±1.20	7.80 ±0.84	7.72 ±0.93	7.66 ±0.74	7.96 ±1.36
RBC (10^{12}/L)	Exper.	5.43 ±0.36	5.67 ±0.25	5.69 ±0.34	5.59 ±0.32	5.71 ±0.36	5.59 ±0.32	5.78 ±0.46	5.76 ±0.32	5.69 ±0.24	5.64 ±0.29	5.72 ±0.40
	Contr.	5.51 ±0.31	5.61 ±0.29	5.73 ±0.31	5.70 ±0.27	5,67 ±0,23	5.67 ±0.24	5,81 ±0.54	5.77 ±0.24	5.86 ±0.31	5.74 ±0.34	5.81 ±050
HGB (g/dL)	Exper.	8.82 ±0.51	8.85 ±0.71	8.90 ±0.65	8.90 ±0.58	9.08 ±0.77	9.17 ±0.66	8.88 ±0.64	8.83 ±0.54	8.88 ±0.71	8.98 ±0.71	8.88 ±0.66
	Contr.	8.67 ±0.80	8.72 ±1.17	8.88 ±1.15	8.73 ±1.32	9,08 ±1,09	8.92 ±0.81	8.75 ±0.92	9.12 ±1.08	9.00 ±0.88	8.88 ±1.26	9.03 ±1.28
HCT (L/L)	Exper.	25.38 ±1.45	25.68 ±1.75	25.35 ±1.71	25.73 ±2.25	26.17 ±2.48	25.55 ±1.89	25.75 ±1.23	25.82 ±1.76	26.00 ±1.80	25.62 ±1.75	25.77 ±2.12
	Contr.	25.90 ±1.65	26.17 ±1.54	26.38 ±1.74	26.22 ±1.94	26,45 ±1,73	25.97 ±1.67	26.37 ±1.72	26.43 ±1.52	26.22 ±1.48	26.38 ±1.61	26.30 ±1.37
MCV (fL)	Exper.	43.13 ±4.06	43.22 ±4.26	43.20 ±4.12	43.47 ±4.17	44.23 ±5.03	43.85 ±4.51	43.90 ±4.04	43.55 ±4.22	43.63 ±4.64	43.73 ±4.48	44.05 ±4.58
	Contr.	43.54 ±3.78	42.68 ±4.51	42.87 ±3.57	44.73 ±3.55	44,00 ±3,18	44.03 ±3.77	44.07 ±3.69	43.48 ±3.74	43.80 ±3.62	44.02 ±3.78	43.95 ±4.06
MCH (pg)	Exper.	16.13 ±1.36	16.07 ±1.58	16.03 ±1.53	16.03 ±1.31	16.27 ±1.60	16.27 ±1.75	16.37 ±1.78	16.57 ±1.80	16.42 ±1.94	16.43 ±1.72	14.62 ±4.88
	Contr.	16.53 ±1.17	16.53 ±1.24	16.33 ±1.28	16.53 ±1.09	16,38 ±1,03	16.42 ±0.94	16.78 ±1.10	16.13 ±0.95	16.48 ±1.53	16.40 ±0.88	16.28 ±1.45
MCHC (g/dL)	Exper.	35.37 ±0.37	35.38 ±0.55	35.50 ±0.53	35.07 ±0.31	35.10 ±0.30	35.32 ±0.73	34.88 ±0.84	34.83 ±0.60	34.65 ±0.72	34.92 ±0.34	34.67 ±0.75
	Contr.	34.95 ±0.57	35.15 ±0.44	34.92 ±0.48	35.25 ±0.64	35,22 ±0,53	35.15 ±0.48	35.22 ±0.70	34.38 ±0.73	34.55 ±0.78	34.58 ±0.74	34.08 ±0.50
PLT (10^9/L)	Exper.	276.67 ±28.37	250.50 ±62.99	261.83 ±43.47	240.33 ±65.08	233.00 ±71.45	267.83 ±89.21	276.00 ±16.27	275.67 ±15.91	311.00 ±54.06	298.50 ±17.07	275.17 ±25.02
	Contr.	277.00 ±37.38	282.33 ±25.02	280.83 ±24.73	252.17 ±35.65	255,67 ±60,58	286.33 ±25.10	274.33 ±16.60	269.83 ±23.61	28333 ±46.68	290.17 ±10.24	283.83 ±14.25

* no statistical difference was noted between pre-treatment values and any of the days after treatment, and there were no statistically significant differences between the groups of cows

Discussion

This is the first study to investigate the effect of selenitetriglycerides on selenium concentrations in cows. The dose of the supplement was determined based on the results of a study which demonstrated the low toxicity of selenite triglycerides in rats (Jastrzębski et al. 1997) as well as the only study evaluating the supplement's effect in sheep (Zagrodzki et al. 2000). In the study by Zagrodzki et al. (2000), sheep were administered 60 mg of Se/animal/day over a period of one month. However, the animals' weight was not reported, and selenium concentrations were not monitored during the experiment. In the current study, the selenium dose was set at 300 mg Se/cow/day, which corresponds to approximately 0.5 mg/kg BW.

The reference values for serum selenium concentration in cattle vary in the literature, probably attributable to differences in the location of the experimental sites. Stowe and Herdt (1992) reported that serum selenium concentrations of 70-100 µg/L were adequate, whereas values in the range of 40-70 µg/L were regarded as marginal. According to Gerloff (1992), blood selenium concentrations below 40 µg/L are indicative of a selenium deficiency. Pavlata et al. (2000) stated that selenium concentrations of greater than 100 µg/L were sufficient, a concentration range of 70-100 µg/L was defined as marginal, whereas cows with less than 70 µg/L were regarded as deficient in selenium. Based on these studies, the serum selenium concentration (64.92 µg/L) prior to supplementation in the present study was deficient.

Selenium supplementation can be toxic. The results of clinical examinations and the information provided by the breeder indicate that selenitetriglycerides did not compromise the health of the cows during the 28-day experiment. In contrast, in a study by Kaur et al. (2005), sodium selenite was administered to cows at 2.5 mg/kg

Table 4. Serum activity of aspartate aminotransferase, creatine kinase, lactate dehydrogenase and gamma-glutamyl transferase in cows with supplementation of selenitetriglycerides and control group (mean+SD).*

		Days										
Parameters		1	2	3	4	5	6	7	14	18	21	28
AST (U/L)	Exper.	70.33 ±5.32	75.50 ±8.69	71.67 ±9.40	71.67 ±5.92	72.33 ±6.62	68.50 ±8.31	71.17 ±7.11	77.50 ±14.63	78.33 ±8.66	72.83 ±9.26	74.50 ±10.82
	Contr.	68,50 ±5,39	73.17 ±7.25	72.00 ±5.21	70.67 ±6.41	71.83 ±5.41	76.50 ±5.32	70.50 ±8.19	74.33 5.23	76.67 ±6.89	71,17 ±7.98	79.00 ±10.82
CK (U/L)	Exper.	125.23 ±17.11	141.40 ±18.35	137.53 ±23.63	129.97 ±9.94	132.65 ±21.72	144.70 ±39.36	136.38 ±24.94	137.17 ±22.36	119.05 ±9.86	141.42 ±43.83	126.83 ±55.52
	Contr.	138,25 ±20,42	138.78 ±18.70	148.60 ±17.76	136.27 ±22.33	133.65 ±12.16	143.60 ±17.87	141.37 ±20.44	142.03 17.22	124.43 ±7.94	150.70 ±31.90	126.22 ±31.90
LDH (U/L)	Exper.	1701.83 ±174.28	1747.17 ±146.14	1718.83 ±174.32	1616.17 ±282.28	1699.00 ±191.75	1580.33 ±226.04	1645.67 ±191.73	1596.67 ±335.93	1602.17 ±261.64	1746.67 ±168.44	1759.50 ±301.84
	Contr.	1780,50 ±182,64	1776.50 ±161.93	1711.17 ±298.43	1698.00 ±231.97	1755.00 ±162.99	1712.33 ±188.39	1587.00 ±319.23	1601.67 297.41	1573.67 ±303.10	1670.17 ±248.10	1795.00 ±162.12
GGT (U/L)	Exper.	32.33 ±4.50	30.83 ±4.22	31.17 ±5.08	31.00 ±4.65	31.67 ±5.16	29.83 ±4.92	30.83 ±5.56	30.50 ±5.75	30.50 ±4.76	30.00 ±4.82	30.67 ±3.44
	Contr.	31,50 ±3,78	29.83 ±5.23	31.33 ±4.46	31.17 ±4.53	32.50 ±4.23	31.00 ±3.58	31.17 ±4.79	31.67 4.88	31.00 ±5.73	30.17 ±4.17	30.17 ±3.43

* no statistical difference was noted between pre-treatment values and any of the days after treatment and there were no statistically significant differences between the groups of cows

Table 5. Serum concentrations of triglycerides, cholesterol, free fatty acids, glucose, total protein, urea and creatinine in cows with supplementation of selenitetriglycerides and control group (mean+SD).

		Days										
Parameters		1	2	3	4	5	6	7	14	18	21	28
TG (mmol/L)	Exper.	0.20 ±0.01	0.18 ±0.02	0.18 ±0.02	0.19 ±0.02	0.19 ±0.01	0.18 ±0.02	0.18 ±0.03	0.21 ±0.01	0.19± 0.02	0.18 ±0.03	0.20 ±0.06
	Contr.	0,20 ±0,01	0.19 ±0.01	0.19 ±0.02	0.19 ±0.01	0.20 ±0.01	0.18 ±0.01	0.18 ±0.02	0.20 ±0.02	0.20 ±0.01	0.18 ±0.03	0.19 ±0.03
Chol (mmol/L)	Exper.	3.06 ±0.60	3.08 ±0.64	3.20 ±0.67	3.05 ±0.71	3.15 ±0.52	3.13 ±0.56	3.15 ±0.62	3.53 ±0.57	3.40 ±0.62	3.29 ±0.62	2.66 ±0.48
	Contr.	3,09 ±0,59	3.52 ±0.58	3.44 ±0.48	3.07 ±0.53	3.13 ±0.47	3.00 ±0.45	3.31 ±0.41	3,23 ±0.46	3.23 ±0.55	3.35 ±0.65	3.17 ±0.40
NEFA (mmol/L)	Exper.	0.36 ±0.16	0.18^{B} ±0.04	0.19^{B} ±0.04	$0.16^{A,B}$ ±0.04	0.15^{A} ±0.03	0.19 ±0.05	0.19^{B} ±0.04	0.18^{B} ±0.03	0.17^{B} ±0.04	$0.15^{A,B}$ ±0.02	$0.14^{A,B}$ ±0.02
	Contr.	0,34 ±0,10	0.35 ±0.11	0.35 ±0.11	0.33 ±0.12	0.30 ±0.16	0.30 0.12	0.32 ±0.10	0.35 ±0.12	0.31 ±0.12	0.32 ±0.09	0.34 ±0.13
Gluc (mmol/L)	Exper.	3.54 ±0.27	3.55 ±0.27	3.50 ±0.40	3.51 ±0.35	3.38 ±0.40	3.52 ±0.20	3.34 ±0.39	3.32 ±0.27	3.48 ±0.23	3.24 ±0.39	3.58 ±0.30
	Contr.	3,55 ±0,27	3.54 ±0.24	3.47 ±0.47	3.30 ±0.29	3.46 0.25	3.50 ±0.20	3.44 ±0.34	3.30 ±0.35	3.36 ±0.33	3.60 ±0.30	3.42 ±0.31
TP (g/L)	Exper.	74.53 ±4.53	75.00 ±3.92	71.33 ±1.21	70.80 ±0.75	71.35 ±1.60	71.17 ±1.17	71.63 ±1.64	72.17 ±2.12	72.52 ±1.57	71.42 ±1.49	76.10 ±7.22
	Contr.	73,98 ±3,53	74.90 ±2.84	73.02 ±2.61	72.53 ±3.02	72.17 ±1.98	72.75 ±1.52	72.58 ±1.41	72.58 ±1.93	72.62 ±1.44	72.52 ±1.43	73.38 ±1.18
Urea (mmol/L)	Exper.	4.85 ±0.35	4.91 ±0.62	4.75 ±0.43	4.61 ±0.35	4.80 ±0.39	4.67 ±0.30	4.45 ±0.21	5.15 ±0.13	4.77 ±0.25	4.92 ±0.23	4.55 ±0.26
	Contr.	4,88 ±0,34	4.87 ±0.30	4.90 0.21	4.68 ±0.23	4.91 ±0.28	4.83 ±0.33	4.67 ±0.20	4.94 ±0.30	4.92 ±0.19	4.80 ±0.08	4.77 ±0.16
Crea (µmol/L)	Exper.	90.47 ±4.46	91.48 ±2.57	91.77 ±2.13	88.77 ±2.65	86.97 ±3.55	88.27 ±4.08	88.35 ±2.28	91.83 ±4.15	92.73 ±2.90	90.05 ±2.81	89.02 ±2.54
	Contr.	88,70 ±3,70	90.65 ±2.59	90.97 ±2.27	92.23 ±2.58	91.53 ±2.13	90.64 ±3.06	89.73 ±3.19	90.70 ±4.49	91.40 ±4.51	91.52 ±3.61	92.62 ±2.84

A – significant differences at P≤0.05 between sampling (day 1 vs. day 2; day 1 vs. day 3; day 1 vs. day 4; day 1 vs. day 5; day 1 vs. day 6; day 1 vs. day 7; day 1 vs. day 14; day 1 vs. day 18; day 1 vs. day 21; day 1 vs. day 28 in experimental and control groups)
B – significant difference at *P*≤0.05 between group, within days

BW/day for 21 days. The first signs of subacute systemic toxicity; anorexia, redness of the eye, diarrhoea, swelling of the joints and the base of the ear, and wound formation in the pastern region, were observed by the sixth day of the experiment and two of the animals died within 18-21 days. Similar results were reported by Kumar et al. (2008) who orally administered 0.1 and 0.25 mg Se/kg BW/day to calves for 12 weeks. Higher doses led to the onset of selenium toxicity. Signs of selenium poisoning were observed when blood selenium concentration exceeded 1680 µg/l. Deore et al. (2005) made similar observations in calves when blood selenium concentrations exceeded 3400 µg/l. In a study by Tiwary et al. (2006), lambs were administered a single ruminal bolus containing sodium selenate (0, 1, 2, 3 or 4 mg Se/kg BW) or selenomethionine (0, 1, 2, 3, 4, 6 or 8 mg Se/kg BW). The animals were observed for 7 days. Sodium selenate doses higher than 2 mg Se/kg BW and selenomethionine doses higher than 4 mg Se/kg BW resulted in tachypnea and/or respiratory distress after minimal exercise.

Selenium from inorganic mineral compounds (sodium selenate) has low availability in the digestive tract of ruminants. Inorganic selenium is broken down by the ruminal microflora into non-available forms that are excreted in faeces. In the present study, serum selenium concentration more than doubled within 24 hours of first administration (a highly significant increase), which indicates that this form of selenium is highly available for ruminants. According to many authors, an increase in dietary selenium concentrations leads to a rise in serum selenium concentration within 2 to 6 days after supplementation (Longnecker et al. 1996, Ellis et al. 1997). In a study of rats, orally, subcutaneously and parenterally administered selenitetriglycerides were highly available, and the highest serum selenium concentrations were reported 2 hours after oral administration and 2.5 hours after subcutaneous administration (Jastrzębski et al. 1997). In the present study, serum selenium concentration peaked on day 7. It exceeded the initial value by more than 4-fold and significantly exceeded the level regarded as adequate for ruminants (Pavlata et al. 2000). According to many authors, short-term oral supplementation with both organic and inorganic selenium does not lead to a satisfactory increase in selenium concentrations (Ortman and Pehrson 1999, Šustala et al. 2003). In one study in ruminants, it required a month of oral selenium supplementation before maximum serum selenium concentrations were achieved (Guyot et al. 2007).

The results of this study indicate that at the tested dose of selenium there was no effect on red blood cell (RBC) parameters or white blood cell (WBC) counts in cows. Shinde et al. (2009) reported similar RBC counts and hemoglobin values in animals that received and did not receive selenium supplements. Pisek et al. (2008) demonstrated that both organic and inorganic Se supplements did not affect WBC counts in sheep. In contrast, Qureshi et al. (2001) observed significantly higher hemoglobin concentrations and RBC counts in buffalos receiving selenium supplements. Kaur et al. (2005) administered two different doses of sodium selenate to cows: 2.5 mg/kg BW/day for 21 days and 0.25 mg/kg BW/day for 16 weeks, and observed that both doses significantly influenced RBC counts, hemoglobin concentrations, hematocrit levels, and total leukocyte counts. In the cited study, significant changes in mean corpuscular volume and mean corpuscular hemoglobin were noted only in cases of chronic selenium poisoning. In the present study, no significant differences in platelet counts were noted. Similar results were reported by Juniper et al. (2006) who did not observe differences in thrombocyte counts in lactating cows receiving different doses of sodium selenate and selenized yeast.

An analysis of liver enzymes in the present study did not reveal changes in the activity of aspartate aminotransferase, lactate dehydrogenase or gamma-glutamyl transferase, which suggests that selenitetriglycerides administered at 300 mg/animal/day did not affect liver function. In a study by Ellis et al. (1997), liver parameters in cows did not change in response to sodium selenate administered at 100 mg/animal/day for 28 days. Bagnicka et al. (2017) did not report changes in AST and GGT activity in cows receiving both organic and inorganic selenium supplements. Kumar et al. (2008) observed increase of serum AST activity in calves after 4 weeks of treatment by sodium selenite in the dosage 0.25 mg per kg body weight. According to Zaki et al. (2018), an increase in AST values is a particularly reliable indicator of selenium's toxic effects on ruminants. In the present experiment, no significant changes in creatine kinase activity were noted, which corroborates the findings of other authors who did not find a correlation between AST values and selenium supplementation (Bagnicka et al. 2017). Creatine kinase is the most sensitive and specific indicator of muscular disorders, and CK activities can range from 1000 to 5000 U/L in affected animals, depending on the severity of the muscular disorder (Radostits et al. 2000). Abutarbush and Radostits (2003) demonstrated that CK activities can be as high as 29,000 U/L in animals with muscular dystrophy caused by selenium deficiency.

In the current study, only minor fluctuations in serum triglyceride concentrations were noted during the experiment. In the literature, selenium supplements did not influence triglyceride concentrations in some studies; in calves (Shinde et al. 2009) or cows (Bagnicka et al. 2017). In contrast, Falkowska et al. (2000) and Kalmath

et al. (2015) found that selenium and vitamin E supplements led to a significant increase in triglyceride concentrations. In the present experiment, serum cholesterol concentrations did not change significantly in response to selenium supplementation. In a study by Falkowska et al. (2000), selenium and vitamin E supplements did not affect cholesterol concentrations in cows in early lactation. Bagnicka et al. (2017) did not report significant changes in cholesterol concentrations in cows administered organic (selenized yeast, *Saccharomyces cerevisiae*) and inorganic selenium (sodium selenite). In the work of Hall et al. (2014), organic selenium supplements administered to dairy cows decreased serum cholesterol concentrations from calving until 48 hours post-partum relative to the control group. According to Kowalik et al. (2012), who administered selenized yeast to heifers, yeasts can alter the ruminal microbiome and, consequently, influence the synthesis of volatile fatty acids. Cholesterol production is determined by the availability of acetate; therefore, selenized yeast can indirectly decrease its synthesis.

The serum concentrations of non-esterified fatty acids in the supplemented cows were highest on day 1 (0.36±0.16 mmol/L) in the present study. This parameter decreased steadily on successive days of the experiment and did not exceed 0.19 mmol/L after day 3. On days 21 and 28, NEFA concentrations were significantly lower than on day 1. In a study by Hall et al. (2014), the administration of selenized yeast to cows in the last 8 weeks of pregnancy did not induce changes in serum NEFA concentrations after calving. Sobiech et al. (2015) did not report changes in post-partum NEFA concentrations when selenium and vitamin E supplements were administered by the parenteral route in the last 5 days of pregnancy. In the present experiment, the decrease in NEFA concentrations on day 3 could be attributed to a decrease in adipose tissue lipolysis resulting from reduced oxidative stress, highly effective absorption of selenitetriglycerides from the digestive tract, a rapid increase in serum selenium concentration, and the powerful antioxidant effects of selenitetriglycerides (Sochacka et al. 2014). This influence of selenitetriglycerides is confirmed by higher NEFA concentrations in the control cows during the entire experiment.

Orally administered selenitetriglycerides did not influence serum glucose concentrations in cows in this study. Similar results were reported by Juniper et al. (2006) and Falkowska et al. (2000). The results of a study performed on human subjects (Jablonska et al. 2016) suggest that selenium could affect glycemic control at different levels of regulation, including insulin signaling, glycolysis and pyruvate metabolism.

The results of this study and the findings of other authors (Juniper et al. 2006, Sobiech et al. 2015, Bagnicka et al. 2017) suggest that selenium supplementation is not correlated with total protein concentrations. According to Kumar et al. (2018), hypoproteinemia in broiler chickens could be attributed to liver degeneration and/or necrosis resulting from the addition of sodium selenate (15 and 30 ppm) to drinking water.

No significant changes in serum urea or creatinine concentrations were observed in the present study, which suggests that selenitetriglycerides do not compromise kidney function. Other authors did not report changes in urea or creatinine concentrations in animals receiving therapeutic doses of selenium supplements (Juniper et al. 2006, Shinde et al. 2009, Bagnicka et al. 2017). An increase in the above parameters could be indicative of kidney damage that accompanies hyperselenosis, and such observations were made by Ahmed et al. (1998) in goats and by Kumar et al. (2018) in birds. Selenium, in particular inorganic selenium, exerts nephrotoxic effects by damaging epithelial cells in proximal and distal convoluted tubules and inducing tubular damage.

Conclusions

The results of this study indicate that orally administered selenitetriglycerides are a highly available and safe source of selenium for cattle. The increases in serum selenium concentrations reported here were much greater than those achieved previously with other selenium supplements in cows. Given the increased bioavailability, safety and efficacy shown in this pilot study, further research is warranted to evaluate the effect of selenitetriglycerides on the immune system, and potential clinical benefits in selenium-deficient cattle.

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