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The effect of inorganic and organic selenium added to diets on milk yield, milk chemical and mineral composition and the blood serum metabolic profile of dairy cows*

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The study was to determine the effect of Se in its inorganic (sodium selenite) compared to organic (selenised yeast, *Saccharomyces cerevisiae*) forms added to a grass- plus maize-silage based diet on milk yield, milk composition and blood serum metabolic profile of dairy cows. The experiment lasted for 90 days between 150 and 240 day of lactation (SD=26) to avoid the burden connected with high milk productivity in the beginning and at the peak of lactation. One group of cows was supplemented with the mixture containing inorganic Se (sodium selenite), while in the organic treatment the mineral-vitamin mixture was identical except for no sodium selenite added, with Se included in the form of selenised yeast (6 g of yeast/cow/day = 6 mg Se/cow/day). After 90 days of the experiment no differences were observed between the treatments in the amount of fodder consumed, daily milk yield or milk components. However, the cows under the Se organic treatment had higher overall milk production (20%). Selenium concentration in milk from the organic selenium treatment was increased by over 300% compared to the inorganic Se treatment. There were no differences in blood

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serum biochemical parameters and the activity of lysosomal enzymes between treatments. Thus, the Se yeast supplementation at 6g per day has no negative effect on the health status of dairy cows. Milk of all cows had a FPD closer to 0°C at 150 vs. 240 days of lactation. It might mean that the organisms still underwent some physiological disturbances associated with high milk production.

KEY WORDS: dairy cattle / selenium / organic / inorganic / productivity

Deficiency of selenium (Se) decreases milk yield and causes an immune deficiency, and therefore increases susceptibility to diseases, including mammary gland disorders in dairy cows [Smith *et al.* 1997]. Therefore, Se supplementation of animal diets is required, especially in the countries where the soils are poor in this trace element [FAO/WHO, 2004]. Currently, Se may be added to diets of cows in the inorganic (sodium selenite and selenate) or organic form (selenised yeast, containing 54–90% Se bound with methionine) [Rayman 2004]. Surai [2006] showed that Se incorporated in yeast is absorbed more efficiently than inorganic Se. Moreover, Se supplied in the inorganic form may have a pro-oxidative effect and show toxicity at excessive concentrations, which is not the case with methionine-bound Se [Seko *et al.* 1989]. The addition of organic Se at a quantity ten times higher (3 ppm in the diet dry matter) than the applicable standards has no negative effect on cow organisms [Stockdale and Gill 2011, Stockdale *et al.* 2011]. Furthermore, the Se content both in the blood plasma and milk was higher when selenised yeast was added to cows' diets compared to sodium selenite [Weiss and Hogan 2005, Juniper *et al.* 2006].

There are some papers concerning the influence of Se yeast supplementation on its concentration in milk, blood, some organs or tissues [Ceballos *et al*. 2009]. To date, however, as summarized by Krzyżewski *et al.* [2014], limited studies have been conducted on the effect of dairy cows' diet supplementation with inorganic vs. organic Se on cows' health. Moreover, contradictory results were obtained concerning productivity of dairy animals [Givens *et al*. 2004; Juniper *et al.* 2006, Bagnicka *et al*. 2016].

Therefore, the aim of the study was to determine the effect of Se in its inorganic (sodium selenite) compared to its organic form (selenised yeast, *Saccharomyces cerevisiae*), added to a grass- plus maize-silage based diet, on milk yield, milk composition and the blood serum metabolic profile.

Material and methods

Cows

The study was conducted on Polish Holstein-Friesian cows of the Black-and-White variety, kept in the experimental barn at Kosów, owned by the Institute of Genetics and Animal Breeding of the Polish Academy of Sciences. From the herd of 120 dairy cows, 16 cows with the body weight of 561 kg (SD=32), in their second lactation were selected for the experiment. The experiment lasted 90 days between 150 and 240 day of lactation (SD=26) to avoid burden connected with high milk productivity in the beginning and at the peak of lactation [Jóźwik *et al.* 2012b]. The animals were divided

into 2 analogous groups of 8 according to their current daily milk yield (DMY=28.4, SD=8.6) and the health status of the mammary gland, assessed on the basis of somatic cell counts in milk (SCC=75.2x10^3/ mL; SD=60.0x10^3/mL) and microbiological tests. Only cows with no subclinical mastitis were selected.

Feed and management

The total mixed ration (TMR) diet was balanced [IZ PIB-INRA, 2009] so that its *ad libitum* intake would ensure welfare and satisfy production needs of the cow, at a milk yield of 30 kg/day, with 4.0% of fat and 3.5% proteins (Tab. 1). Cows of both treatments were given identical basal feed rations. All cows in the herd are routinely supplemented with a mineral-vitamin mixture (VITAMIX KW, Polmass, Poland) added to the TMR.

Table 1. Chemical composition, feeding value and concentrate mixture components

Component	Dry matter (%)	In dry matter					
		crude protein (%)	crude fibre (%)	PDIE (g kg^{-1})	PDIN (g kg^{-1})	UFL (units)	%
Corn silage	31.5	8.79	22.74	88	72	0.88	37.1
Wilted grass silage	38.0	13.26	33.75	83	76	0.70	17.9
Barley straw	86.4	3.26	42.30	45	23	0.41	2.0
Corn grain silage	65.2	11.03	13.49	120	83	1.22	12.3
Concentrate mixture	78.9	21.19	6.00	132	166	0.98	30.7
Concentrate mixture	87.1	39.10	12.00	163	271	1.02	18.0
rapeseed extracted meal							
triticale	87.0	10.72	3.12	106	89	1.01	51.6
soya extracted meal	85.1	41.28	11.10	242	354	1.13	20.0
ketomix E 18[/1]	47.1	13.00	-	-	-	-	2.7
chalk	-	-	-	-	-	-	2.0
co-bind A-Z[/2]	-	-	-	-	-	-	0.5
sodium bicarbonate	-	-	-	-	-	-	2.4
vitamix KW[/3]	-	-	-	-	-	-	2,8
whole diet (TMR)	49.0	15.0	19.4	102	108	0.94	100.00

UFL – 7200 kcal NEL, NEL – net energy for lactation, UFL – feed unit for lactation, PDIN – protein digested in the small intestine supplied by rumen-undegraded dietary protein and by microbial protein from rumen-degraded organic matter, PDIE – protein digested in the small intestine supplied by rumen-undegraded dietary protein and by microbial protein from rumen-fermented organic matter.
[1]Energy supplement (3.36 UFL/kg DM).
[2]Nneutraliser of mycotoxins; 3 - macroelements (g/kg): Ca 120, P 60, Mg 50, Na 110; microelements (mg/kg): Mn 4000, Zn 9500, Cu 1150, Iodine 90, Co 25, Se 45; vitamins (mg/kg): E 4000, K3 50, B1 150, B2 100, B6 50; calcium pantothenate 300, niacin 2500, folic acid 30; vitamin A 1000 U/L, D3 120 000 U/L, B12 550 mcg/kg.

In the experiment one group was supplemented with the mixture containing inorganic Se (sodium selenite – SS), while in the organic treatment the mineral-vitamin mixture was identical except for sodium selenite (specially prepared for this experiment by Polmass, Poland), and Se was included in the form of selenised yeast (SY) (6 g of yeast/cow/day = 6 mg Se/cow/day) (Sel-Plex 1000, Alltech, Poland). The Sel-Plex was given individually to the cows by pouring it into the manger on top on the previously

given TMR feed, and the whole amount was consumed by cows, while the intake of SS depended on the amount of feed intake and ranged between 7.43 and 5.88 mg Se/cow/day on average at the beginning and end of the experiment, respectively.

The cows were kept in a free-stall system, in a separate barn equipped with mangers (Calan Gates; American Calan), which allow for the precise, electronic control of feeding of each cow throughout the period of the experiment; each cow had access to only one manger it was assigned. The amounts of feed intake were electronically recorded. All feed ration components were mixed using an experimental SDR mixing fodder wagon (Super Data Ranger; American Calan) to ensure fodder uniformity. The TMR mix was given in such quantities so as to leave at least 5% orts on as-fed basis, calculated on the dry matter. The orts were removed and weighed once daily, and a representative sample proportional to their quantity was collected to form an aggregate sample for further analyses. Representative samples of feed and orts were collected every week and stored at -20°C. The aggregate samples were analysed every 2 weeks.

Milk and blood sample collection

The cows were milked twice daily, at 6 a.m. and 6 p.m. The milk yield of each cow was individually and electronically recorded at each milking. In order to analyse the milk component contents, representative morning milk samples were taken individually from each cow twice: on the day before the start and on the last day of the experiment. Milk samples for chemical composition analysis were collected to polypropylene containers with a preservative (Microtabs II, Bentley, Poland). For microbiological examination the foremilk samples, collected in a sterile manner just before morning milking, before the start and at the end of the experiment, were streaked (100 µl) on Columbia agar supplemented with 5% sheep blood (bioMerieux, Craponne, France) and on MacConkey agar (bioMerieux, Craponne, France). Plates were incubated at 37°C for 48 h. The isolated bacteria were identified using VITEC2™ – compact 15, system 04.02 (bioMerieux, USA). Blood samples for the determination of biochemical parameters and lysosomal enzyme activity in blood serum were collected from the jugular vein before the morning feeding by an authorized veterinarian before the start and at the end of the experiment.

Chemical analyses of feed and milk

The contents of basic constituents in feed samples were evaluated using standard methods [AOAC, 2000]. Based on the chemical composition, the nutritional values of the feeds were assessed according to the INRA system using the PrevAlim 3.22 software (Version professionnelle intégrale, INRA 1988-2004, France). The SCC with an I**B**C_M device (Bentley Instruments, USA), and several parameters of milk (fat, total protein (TP), casein, lactose, total solids (TS), solids non-fat (SNF), citric acid, free fatty acids (FFA) contents, urea level, acidity and freezing point) were evaluated using a MilkoScan FT2 device (Foss Analytical, Denmark). The blood serum biochemical parameters (albumins, alkaline aminotransferase, asparagine

aminotransferase, bilirubin, calcium, cholesterol, creatinine kinase, chlorine, creatinine, gamma-glutamyltransferase, glucose, iron, potassium, cholesterol LDL, hepatic lipase, magnesium, sodium, phosphorus, total protein, triglycerides) were estimated using the COBAS INTEGRA®400 plus system (Roche Diagnostics Ltd., Rotkreuz, Switzerland). Macroelements (sodium – Na, potassium – K, calcium – Ca, magnesium – Mg) and microelements (zinc – Zn, aluminum – Al, vanadium – V, chromium – Cr, manganese – Mn, iron – Fe, cobalt – Co, nickel – Ni, copper – Cu, arsenic – As, selenium – Se, strontium – Sr, cadmium – Cd) in milk were determined by inductively coupled plasma-atomic emission spectrometry using ICP-MS [Enamorado-Báez *et al.* 2013].

The activities of alanyl-aminopeptidase (AlaAP - E.C. 3.4.11.2), leucylamino-peptidase (LeuAP - E.C. 3.4.11.1), arginyl-aminopeptidase (ArgAP - E.C. 3.4.11.6), acid phosphatase (AcP - E.C. 3.1.3.2), α-glucuronidase (BGRD - E.C. 3.2.1.31), β-galactosidase (BGAL - E.C. 3.2.1.23), β-glucosidase (BGLU - E.C. 3.2.1.21), α-glucosidase (AGLU E.C. 3.2.1.20) and N-acetyl-beta-glucosaminidase (NAG, E.C. 3.2.1.30) were determined in the lysosomal fractions of blood serum. The activity of AcP, BGRD, BGAL, BGLU, AGLU, and NAG were measured as 4-nitrophenyl derivatives at 420 nm according to the micromethod of Barrett and Heath [1972]. The activity of AlaAP, LeuAP and ArgAP were measured as Fast Blue BB salt (4-benzoyloamino-2, 5-diethoxybenzenediazinium chloride) derivatives at 540 nm by the method of McDonald and Barrett [1986]. In the blood serum protein concentration was measured by the modified Total Protein-Biuret Method [Krawczyński and Osiński 1967].

Statistical analysis

The obtained results were analysed using the analysis of variance. The GLM procedure with the Bonferroni adjustment of the SAS [SAS/STAT 2002-2010] was used. The statistical model consisted of the fixed effect of the treatment and the time of sample collection, as well as their interaction. The model for the analysis of component contents in milk also included the daily milk yield as a linear covariate. Prior to analysis, all traits were tested for normality of distribution and SCC was transformed to natural logarithm values. The Pearson correlation coefficients between mineral elements in the organic treatment at the end of experiment were estimated using the CORR procedure of SAS package programs [SAS/STAT 2002-2010].

Results and discussion

After 90 days of the experiment no differences were observed between the treatments in terms of the daily amount of fodder consumed, daily milk yield, and daily yield of milk components (Tab. 2) or in the component contents (Tab. 3). However, the analysis of total milk yield for the whole period of the experiment showed that the quantity of milk produced by cows supplemented with SY was higher (Tab. 2). The milk of cows treated with both SY and SS had a freezing point closer to 0°C at the beginning of the experiment.

Table 2. Least squares means of dry matter intake, daily milk yield and milk components, and milk yield during the experiment with their standard errors (SEM)

Parameter	Inorganic treatment		Organic treatment		SEM
	initial period	day 90 samples	initial period	day 90 samples	
Body weight (kg)	550	554	571	570	11
Dry matter (DM) intake (kg)	19.2	15.2	19.8	14.8	1.40
Milk yield (kg)	27.9	20.5	28.8	22.4	2.50
Value corrected milk – VCM* (kg)	32.7	25.2	32.7	27.5	2.50
Total solids – TS (kg_	3.59	2.73	3.66	3.01	0.33
Solids non-fat – SNF (kg)	2.60	1.90	2.67	2.10	0.20
Fat yield (kg)	1.08	0.87	1.08	0.97	0.16
Protein yield (kg)	0.95	0.72	0.95	0.79	0.06
Lactose yield (kg)	1.36	0.99	1.39	1.07	0.10
Casein yield (kg)	0.71	0.56	0.70	0.61	0.05
Milk yield throughout experimental period (kg)	1767[a]		2211[b]		118

[ab]Within rows means bearing different superscripts differ significantly at p≤0.05.
*VCM (kg) = 0.05 x milk yield (kg) + 8.66 x fat (kg) + 25.98 x protein (kg) [Arbel *et al*. 2001].

Table 3. Least squares means of milk chemical composition, acidity, freezing point, urea content and lnSCC with their standard errors (SEM)

Parameter	Inorganic treatment		Organic treatment		SEM
	initial period	day 90 samples	initial period	day 90 samples	
Fat (%)	3.78	4.38	3.47	4.34	0.35
Total protein – TP (%)	3.55	3.46	3.41	3.50	0.12
Casein (%)	2.65	2.67	2.52	2.70	0.10
Lactose (%)	4.84	4.83	4.84	4.78	0.08
Total solids, %	12.89	13.43	12.49	13.40	0.39
Solids non-fat – SNF (%)	9.40	9.23	9.33	9.32	0.11
Citric acid (%)	0.15	0.20	0.16	0.20	0.01
Free fatty acids – FFA (%)	0.84	0.66	0.99	0.61	0.12
Urea (mg/L)	200	218	190	228	17
Acidity[1] (in Turner's degrees)	17.69	18.34	17.55	18.68	0.66
LnSCC[2]	4.21	4.09	4.29	4.35	0.50
FPD[3] (°C)	-0.540[A]	-0.567[B]	-0.539[A]	-0.563[B]	0.004

[AB]Within rows means bearing different superscripts differ significantly at p≤0.01.
[1]Fresh milk acidity is 16°–18°T (Turner's degrees).
[2]LnSCC – the natural logarithm of the number of milk somatic cells.
[3]FPD – freezing point depression.

In our study no influence of the Se form added to the cows' diet on the milk somatic cell count was observed (Tab. 3). However, it should be stressed that the milk of cows selected for the experiments was characterized by low SCC in both groups (between $12x10^3$ and $173x10^3$). In the mammary glands of these animals there was no subclinical mastitis, as the presence of pathogenic bacteria was not observed

in milk; only the bacteria from the coagulase-negative staphylococci group were present (*Staphylococcus chromogenes*, *Staphylococcus simulans*, *Staphylococcus epidermidis*), that is, environmental and opportunistic bacteria were identified, with the frequency of about 50% for both treatments at the end of experiment.

There were no differences in the level of any studied macro-elements (Tab. 4); however, there were differences between treatments in the level of some microelements (Tab. 5). As expected, the addition of SY contributed to an increase of Se concentration in milk. After 90 days of the experiment its content in the SS treatment was 0.990 µg/L, compared to 3.065 µg/L in the SY treatment. Apart from Se, higher levels of

Table 4. Least squares means of contents of basic macroelements in milk (mg/100g^{-1}) with their standard errors (SEM)

Parameter	Inorganic treatment		Organic treatment		SEM	Range of content in milk
	initial period	day 90 samples	initial period	day 90 samples		
Na	44.5	51.2	54.1	49.5	5.6	50-60 mg 100g^{-1}
Mg	11.0	11.7	11.0	12.6	1.5	9-13 mg 100g^{-1}
K	103.2	120.1	114.4	135.9	12.2	140-160 mg 100g^{-1}
Ca	112.9	121.3	107.7	148.2	13.2	110-125 mg 100g^{-1}

Table 5. Least squares means of contents of selenium and other essential microelements in milk

Parameter	Inorganic treatment		Organic treatment		SEM	Average or range of content in cow milk
	initial period	day 90 samples	initial period	day 90 samples		
Zn mg 100g^{-1}	0.221^{a}	0.288^{a}	0.365^{a}	0.630^{b}	0.080	0.5 mg 100g^{-1}*
Al µg mL^{-1}	0.273	0.319	0.380	0.411	0.027	0.8 µg mL^{-1}**
V ng g^{-1}	0.312^{A}	0.256^{A}	0.224^{A}	0.624^{B}	0.064	25.6-42 ng g^{-1} dry weight***
Cr µg L^{-1}	11.13	13.98	5.63	8.63	1.650	5-50 µg L^{-1}*
Mn µg L^{-1}	1.493^{A}	1.711^{A}	1.521^{A}	2.720^{B}	0.274	0.02 µg 100g^{-1}*
Fe mg 100g^{-1}	0.052	0.045	0.062	0.073	0.001	0.04 mg 100g^{-1}*
Co µg L^{-1}	0.38	0.54	0.48	0.66	0.100	0.4-1.1 µg L^{-1}*
Ni µg L^{-1}	29.27	25.62	24.02	24.28	7.860	26 µg L^{-1}*
Cu mg 100g^{-1}	0.004	0.003	0.004	0.005	0.001	0.01 mg 100g^{-1}*
As ng g^{-1}	27.28	32.45	22.45	30.58	3.590	10 ng g^{-1}*
Se µg L^{-1}	0.689^{A}	0.990^{A}	0.120^{A}	3.065^{B}	0.275	0.96 µg 100g^{-1}*
Sr ng g^{-1}	760^{a}	1344^{a}	1208^{a}	2356^{b}	416	1910-3150ng g^{-1} dry weight***
Cd mg L^{-1}	0.006	0.007	0.007	0.010	0.002	0.7-23.1 mg L^{-1}*

^{AB}Within rows means bearing different superscripts differ significantly at: small letters – p≤0.05; capitals – p≤0.01.
*Park *et al*. [2013]; **Viñas *et al*. [1997]; ***Ataro *et al*. [2008].

Zn, V, Mn and Sr were found in the animals in the SY treatment at the end of the experiment when compared to the start of the experiment and to the SS treatment. The high correlation coefficients between the Se content in milk and the concentrations of Zn and Sr, and V were found at the beginning of the experiment (Tab. 6a). High or medium correlation coefficients were found between several other microelements as well. There was no correlation between Se and other trace elements at the end of the experiment in either of the treatments (Tab. 6b and 6c). However, positive

Table 6a. Pearson's correlation coefficients between concentrations of microelements at the beginning of experiment (including of both group of treatments)

Item	Zn	Sr	V	Al	Cd	Ni	Ca	Cr
Se	0.65*	0.55*	-0.56*					
Zn	-	0.92**			0.68*			
Sr	0.92**	0.55*		0.65*	0.58*			
K				0.60*		-0.78**		
Na							0.62*	
Mg	0.53*	0.59*					0.72**	
Co				0.65*			0.64*	
Cu								-0.62*

*P≤0.05; **P≤0.01.

Table 6b. Pearson's correlation coefficients between concentrations of microelements at the end of experiment in inorganic treatment

Item	Zn	Sr	V	Ni	Ca	Cr
Al				0.99**		
Mo	0.93*				0.96*	
Mg					0.94*	
K			-0.98*			
Mn		0.94*				-0.96*
Cu	0.96*					

*P≤0.05; **P≤0.01.

Table 6c. Pearson's correlation coefficients between concentrations of microelements at the end of experiment in organic treatment

Item	Sr	Cd	Ni	Cr	K
Zn		0.84*	0.92**		-0.82*
Sr		0.92**	0.92**		
Mg			0.79*		
Co	0.84*		0.79*		
Cd			0.82*		
Mn	0.77*	0.82*	0.85*	-0.82*	

*P≤0.05; **P≤0.01.

correlation coefficients were found between the Zn, Mn, and Sr concentrations and the concentrations of Cd and Ni in SY treatment (Tab. 6c).

The absence of any negative effect of used additives on the health of cows is also confirmed by the basic markers of the metabolic profile (Tab. 7) and activity of lysosomal enzymes (Tab. 8). There were differences in the activity of one out of six studied glycosidases (BGRD) and in the activity of three out of four studied aminopeptidases (AlaAP, LeuAP, ArgAP) between the start and the end of the experiment in both SS and SY treatments, without differences between treatments (Tab. 8).

Results of the presented study showed no differences in daily milk yield and its composition and indicate a similar nutritional value and a comparable degree of nutrient utilisation in both diets. However, the quantity of milk produced by cows supplemented with SY for the whole period of the experiment was higher by about 20% (Tab. 2), despite the small number of cows in the treatments and the short duration of the experiment. Therefore, thanks to the supplementation with SY throughout the whole lactation period the milk yield is likely to be much higher when compared to the yield of animals supplemented with SS. Even more so, since the results of a parallel experiment, conducted throughout the whole lactation period on dairy goats kept on the same farm, indicate an increased average daily milk yield [Bagnicka *et al.* 2016] and the expression of the *CSN1S2* gene (alpha-s2 casein) as well [Bagnicka *et al*. 2015] due to diet supplementation with SY compared to SS, even though the animals of both species on that farm are routinely administrated a mineral-vitamin mixture. Moreover, Wang *et*

Table 7. Least squares means of parameters in blood serum and their standard errors (SE)

Item	Inorganic treatment		Organic treatment		SEM	Reference values [Winnicka *et al.* 2004]
	initial period	day 90 samples	initial period	day 90 samples		
Albumin (g L^{-1})	37.9	38.0	39.2	39.3	0.9	27-43
Alkaline aminotransferase (UL^{-1})	24.5	24.6	24.9	26.9	1.6	17-37
Asparagine aminotransferase (U/L^{-1})	90.3	92.2	108.9	103.9	10.7	48-100
Bilirubin (μmol L^{-1})	1.35	1.10	1.30	0.83	0.15	1.7-51
Calcium (mmol L^{-1})	2.63	2.44	2.51	2.50	0.08	1.98-2.50
Cholesterol (mmol L^{-1})	5.12	4.49	4.71	4.82	0.46	2.3-6.6
Creatinine kinase (U/L^{-1})	115.8	113.0	118.4	113.0	11.1	44-228
Chlorine (mmol L^{-1})	97.6	95.9	98.9	95.2	1.1	96-104
Creatinine (μmol L^{-1})	68.7	73.8	71.6	73.2	3.4	62-97
Gamma-glutamyl transferase (U/L^{-1})	33.3	34.3	34.1	33.2	2.1	20-48
Glucose (mmol L^{-1})	4.22^{A}	3.50^{B}	4.38^{A}	3.65^{B}	0.04	2.1-3.9
Iron (μmol L^{-1})	26.8	25.7	28.2	26.4	1.7	10-29
Kalium (mmol L^{-1})	4.48	4.29	4.83	4.12	0.19	4.0-5.3
Cholesterol LDL)mmol L^{-1})	1.13	0.90	1.01	1.05	0.19	?
Hepatic lipase (U/L^{-1})	6.40	7.54	6.27	7.61	0.42	?
Magnesium (mmol L^{-1})	0.93	0.99	1.00	0.97	0.04	0.7-1.1
Sodium (mmol L^{-1})	140.8	139.8	140.3	140.1	1.2	139-144
Phosphorus (mmol L^{-1})	1.87	1.82	2.08	2.01	0.14	1.5-2.9
Total protein (g L^{-1})	72.3	68.3	70.3	68.5	1.0	59-77
Triglycerides (mmol L^{-1})	0.14	0.10	0.14	0.12	0.01	0.0-0.2

^{AB}Within rows means bearing different superscripts differ significantly at: small letters – $p \leq 0.05$; capitals – $p \leq 0.01$.
U/L – international unit per liter (unit is an arbitrary amount agreed upon).

Table 8. Least squares means of the activity of lysosomal enzymes and their standard errors in blood serum

Trait	Inorganic treatment		Organic treatment		SEM
	initial period	day 90 samples	initial period	day 90 samples	
AlaAP	1.84^{A}	2.08^{B}	1.83^{A}	2.08^{B}	0.03
LeuAP	1.80^{A}	2.11^{B}	1.85^{A}	2.10^{B}	0.03
ArgAP	1.77^{A}	2.09^{B}	1.86^{A}	2.10^{B}	0.04
AcP	2.22	2.21	2.21	2.21	0.02
BGRD	2.09^{a}	1.92^{b}	2.08^{a}	1.91^{b}	0.04
BGAL	2.07	2.08	2.00	2.07	0.05
BGLU	2.04	2.09	2.05	2.10	0.03
AGLU	2.02	1.94	2.07	1.95	0.05
NAG	1.96	2.08	2.03	2.09	0.06

^{AB}Within rows means bearing different superscripts differ significantly at: small letters – $p \leq 0.05$; capitals – $p \leq 0.01$.
AlaAP – alanyl-aminopeptidase; LeuAP – leucylaminopeptidase; ArgAP – arginyl-aminopeptidase; AcP – acid phosphatase; BGRD – α-glucuronidase; BGAL – β-galactosidase; BGLU – β-glucosidase; AGLU – α-glucosidase; NAG – N-acetyl-beta-glucosaminidase.

al. [2009] also showed that SY contributed to an increase of milk yields. According to those authors, the optimum dose of SY was 300 mg/kg of dry matter. In the presented study the animals received the same amount of SY, calculated as the dry matter intake (6g of Se yeast = 6 000 mg/20 kg DM of feed = +/- 300 mg/kg). As it was shown by Wang *et al.* [2009], the addition of SY improved milk yields thanks to favorable rumen fermentation and feed digestion. Chaucheyras-Durand *et al*. [2008] pointed out that active dry yeast stabilised the ruminal pH, improved fiber degradation and nitrogen metabolism. As revealed by Rao *et al.* [2001], the deficiency of Se in mouse diet led to a higher expression of genes involved in DNA damage, oxidative stress and cell cycle control, at a simultaneous lower expression of genes involved in detoxification processes when compared to the diet supplemented with high levels of Se (1 mg/kg, as seleno-L-methionine). Furthermore, active dry yeast added to the ruminant diet (100 g/day/cow and 10 g/day/goat) stabilised the rumen environment and influenced the expression of several genes associated with the immune system, which may play a role in maintaining homeostasis in the mammary gland [Kowalik *et al*. 2012, Jarczak *et al*. 2014]. As demonstrated by Tripathi and Karim [2010] in a study on lambs, yeast promotes rumen microbial growth and improves degradation of short chain polysaccharides in micro-organisms. Therefore, higher productivity of cows in the case of the SY treatment might be connected with the contribution of both the organic Se form and *S. cerevisiae* to the improved rumen environment and gene expressions.

However, the results obtained in a majority of studies show that neither the form of Se nor the quantity of the additive exerted any significant influence on the milk yield nor fat, protein, or lactose content [Givens *et al.* 2004, Juniper *et al.* 2006, Calamari *et al.* 2011, Stockdale and Gill 2011, Stockdale *et al*. 2011]. Nevertheless, it needs to be remembered that those studies were conducted for only several weeks.

The freezing point depends on the amount of soluble substances in milk; however, feeding and the physiological condition of animals also affect this parameter [Henno *et al.* 2008]. The basic nutrition was the same for all cows, thus changes in the milk freezing point indicate changes in the physiological status of the cows during lactation. The experiment started after the peak of lactation (150 day); however, the freezing point closer to 0°C at this stage of lactation might indicate that the organisms still underwent some physiological disturbances associated with milk production remaining high after the peak of lactation.

The frequency of occurrence of environmental bacteria in milk was the same for both groups at the end of experiment. These results are consistent with those reported by other authors, both at the lesser scale of a single experiment [Calamari *et al*. 2010] and in broader-scale experiments [Ceballos-Marquez *et al*. 2010]. Yatoo *et al*. [2013] also indicated a positive effect of Se on somatic cell counts in bovine milk. Indeed, it is the system of cow maintenance and environmental conditions that play the most important role in maintaining health of the mammary gland. Furthermore, the results of the above-mentioned parallel experiment, conducted throughout the whole lactation period on dairy goats, also indicate a decreased SCC in milk due to supplementation

with selenium in the organic vs. inorganic form [Bagnicka *et al*. 2016]. However, as it was pointed out above, the animals chosen to this experiment had no mammary gland health disorders.

In the organic treatment the Se concentration increased by over 300% compared to the inorganic Se treatment. Such a phenomenon was recorded in all the studies which compared these two forms of Se [Givens *et al.* 2004, Weiss 2005, Juniper *et al*. 2006]. According to Juniper *et al*. [2006] and Doyle *et al*. [2011], there is a linear relationship between the amount of organic Se added to the diets and Se contents in the milk, blood, urine, and faeces of the animals. This relationship was also confirmed by Stockdale and Gill [2011], who used SY added at 20-60 mg/day and recorded a linear increase of Se concentration within 80-399 μg/L. Moreover, Se administered as microencapsulated SS at 0.3 mg/kg of diet dry matter passes to milk in a similar quantity as the same dose of Se in yeast [Grilli *et al.* 2013]. In turn, Se applied at 0.5 mg/kg of diet dry matter in the form of SS microcapsules contributed to a higher Se concentration in cows' blood and milk compared to SY supplementation. Previously, Ortman and Pehrson [1999] indicated that it may be difficult to produce milk with the optimal Se concentration for consumers when using sodium selenite, taking into consideration the dietary Se limits in the USA and the EU (0.3 and 0.5 mg Se/kg of diet dry matter, respectively).

The difference in Se concentration in the milk of cows supplemented with Se in the unprocessed inorganic or organic forms depends on the varying doses, in which this microelement is used. Calamari *et al*. [2010] demonstrated that as little as 3.2% of Se added to diets in the form of SS was transferred to milk, whereas Givens *et al*. [2004] estimated this factor as 9.9-12.5%. Calamari *et al*. [2010] stated that when SY was added to the diet of cows, the transfer of Se to milk was 16.3%. Ceballos *et al*. [2009] showed that the level of Se transfer from the supplements depends on milk yield, diet dry matter intake and lactation stage. The increased absorption of Se from selenised yeast is related to the difference in the absorption from the intestine, the way it is metabolised once absorbed and the preference for the uptake of Se coming from various sources by the mammary gland [Calamari *et al*. 2010]. In selenised yeast, the predominant form is methionine-bound selenium, which constitutes approx. 63% of total Se. The organism of a cow is unable to synthesise SeMet from inorganic Se. Selenium is better absorbed by the organism when it is incorporated in the milk protein together with methionine. Thus the factors influencing the synthesis of milk proteins also affect Se concentration in milk. Calamari *et al.* [2010] reported that in the cows receiving SY it is SeMet that contributes to the increase of Se concentration in milk by 60.3%, while the contribution of selenocysteine (SeCys) is only 5.2-6.3%. Ceballos *et al*. [2009] showed that the addition of SY contributed to a greater extent to the decrease of its concentration in milk compared with selenium added in the same quantity, but in the inorganic form. Studies on pigs have demonstrated that the bioavailability of Se incorporated into the amino acids of milk proteins is significantly higher than that of Se supplied in the inorganic form [McIntosh *et al.* 2008] and that it was more efficient in preventing chemically induced cancer in mice [Hu *et al.* 2008].

The increased Se concentration in milk and milk products is an interesting issue from the point of view of meeting the recommended daily allowances for consumers. The daily requirement of an adult for Se is 55 μg/d [Scientific Committee for Food, 1993], while regarding the prophylactic effect of Se the required intake should be 150-200 μg/d [McIntosh *et al*. 2008]. Stockdalle and Gill [2011] and Stockdalle *et al*. [2011] used very large doses of Se in the diet of cows in order to determine the tolerance of animals to these doses and the maximum possible Se enrichment of milk used for preventive protection of consumers' health. It was shown that the quantity of Se in organic compounds fed to cows may be even 50 times higher than the quantity of this element necessary to meet the requirement of the animals [Stockdale *et al.* 2011, Walker *et al*. 2010].

Some trace elements serving as cofactors of many enzymes play an important role in physiological processes and contribute to maintaining homeostasis of the organisms as well as improving growth and productivity of animals [Jiakuia and Xiaolong 2004, Balicka-Ramisz *et al*. 2006, Enjalbert *et al*. 2006, Poławska *et al.* 2016], including milk yield [Yatoo *et al.* 2013]. In our study increased contents of Zn, V, Mn and Sr were also found in the animals in the organic treatment at the end of the experiment. Improved bioavailability of Zn, Cu and Co was demonstrated by Abdelrahman and Hunaiti [2007], when lambs were supplemented with yeast and cyc-methionine. Moreover, at the beginning of the experiment high correlation coefficients were found between Se content in milk and concentrations of some microelements (positive with Zn and Sr, and negative with V) (Tab. 6a), playing a significant role in the metabolic processes of organisms. In our study no correlation was observed between Se and other trace elements, while high correlations were found between Ni and Cd, and other elements in the organic treatment at the end of experiment. Björklund *et al*. [2012] obtained opposite results to ours in a study on random samples of human milk, as no correlation was reported between Se and Zn, Sr, or V, except for a positive and moderate correlation coefficient between Cd and Mn (0.38). Unfortunately, they did not determine the Ni concentration. However, Lutfullah *et al*. [2014] found a similar relationship between mineral elements in various commercially available powdered and liquid milks.

What is less favorable, is the positive relationship between increased Zn, Mn, and Sr concentrations and the concentrations of Cd and Ni in the SY treatment (Tab. 6c). However, the increased concentrations of Cd and Ni in the SY treatment were not significantly confirmed, probably because of the high variability of these microelement contents (Tab. 5). Moreover, the target concentration values of these toxic metals were significantly lower than the maximum authorized concentrations of these elements in cows' milk [Vińas *et al.* 1997, Park and Haenlein 2013].

Regardless of the Se form added to the diet, the values of most physiological and biochemical indicators fell within the recognized reference ranges. This is also confirmed by the results of Juniper *et al*. [2006], which indicate that equal amounts of Se in the form of SS and SY cause no differences either in milk yield or in the chemical composition of blood and hematological marker values. However, calcium

and glucose contents and AST activity were higher, while bilirubin content was lower than the references values, especially at day 150 of lactation. A high level of glucose indicates stress or pancreatic disorders, while the high activity of AST may be caused by viral hepatitis, toxic liver damage, or metabolic disorders, and reflects disturbances in liver functioning [Djoković *et al*. 2013, http://www. 9sites.org/pigcare/bloodwork.htm). Simultaneously, the low level of bilirubin, which plays an important role as a cellular antioxidant [Sedlak *et al*. 2009], may indicate a low oxidative status of the animals. Apparently, even though the study began after the peak of lactation the productivity of almost 30 kg/day/cow remains very high and still creates a heavy burden for the organism, causing oxidative stress and metabolic disorders [Jóźwik *et al*. 2012a].

Lysosomal enzymes play a critical role in metabolism of carbohydrates and peptides, and their activity is crucial for the functioning of organisms. The deficiency of lysosomal enzymes causes organ malfunction [Meikle *et al*. 1999]. The lack of differences between treatments means that it was the stage of lactation, and not the form of Se supplementation that affected the activity of lysosomal enzymes. The activities of three aminopeptidases were higher, while the activity of glycosidase was lower at the end of experiment. The increase in the activity of aminopeptidases suggests an increase in protein degradation and may also suggest an overabundance of protein in the diet at the end of lactation. On the other hand, glycosidases catalyse the hydrolysis of glycosidic linkages in polysaccharides and glycosylamines (proteoglycans). Some authors suggested that if animals are fed a diet with an insufficient protein level, an increase of glycosidase activity may occur as an adaptation of the organism to unfavourable environmental conditions (protein deficit) [Jarczak *et al*. 2012]. The lower level of aminopeptidases and the higher level of glycosidase at the start and then at the end of the experiment may also suggest insufficient levels of protein in the diet at day 150 of lactation despite *ad libitum* feeding. It was probably the protein-energy balance which had not been provided.

The SY treatment does not disturb homeostasis of the organism, thus, thanks to SY application in the cow diet it is possible to improve lactation milk yield with no adverse effect on metabolism and to provide milk with an elevated content of selenium for consumers. The organisms on 150 day of lactation still underwent some physiological disturbances associated with high milk production.

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