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# Selenium Content and Distribution in Cow's Milk Supplemented with Two Dietary Selenium Sources

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The effect of two sources of Se, selenized yeast (Se-Y) and sodium selenite, added to total mixed rations (TMR) fed to cows on Se milk content and distribution in milk components was studied on three farms for 6 weeks. The maximal increase in milk Se was attained with Se-Y supplemented at  $0.3 \,\mu g \, g^{-1}$ . The effect was immediate, with an increase of  $9 \,\mu g \, L^{-1}$  being observed after only 5 days, and remained steady until the last sample at day 40 of Se supplementation. Se distribution in milk components was constant, 53.6, 42.6, and 9.3% in whey, casein, and fat, respectively, and was unaffected by the form of supplementation. The effect of the level of Se-Y supplementation on milk Se was studied on two farms. Increasing dietary Se-Y from 0 to 0.5  $\mu g \, g^{-1}$  elevated milk Se content from 20 to 39  $\mu g \, L^{-1}$ . Se-enriched cow's milk at different levels can be produced by varying dietary Se supplementation in the form of selenized yeast.

KEYWORDS: Selenium; cow's milk; selenium distribution; selenium supplementation; HGAAS

# INTRODUCTION

Selenium plays an important role in human health as it can have a chemopreventative role in certain forms of cancer (1-3) and has a beneficial effect on a number of pathologies such as infertility (4-6) and hypothyroidism (7). The enzyme glutathione peroxidase and other reductases, which have an antioxidant activity in intracellular reactions, are known to exhibit anticarcinogenic properties (8). Moreover, glutathione peroxidase is important for removing peroxide and other oxidants, due to the reducing behavior of glutathione (9). Recognition of the metabolic importance of selenoproteins helps to explain the adverse consequences of selenium deficiency observed in human health.

Selenium is introduced into the food chain by plants, which absorb inorganic selenium salts from the soil and convert them into organic forms of the element (mainly as selenomethionine), which are then incorporated, nonspecifically, into proteins. However, specific incorporation into selenoproteins, some of which have important enzymatic functions, has been widely reported (9-11).

The concentration of selenium in plants varies widely and depends on the selenium content and characteristics of the soil. In addition, selenium deficiency in plants has been identified in several countries, such as China, Russia, and New Zealand (12). Besides, acid soils and complexation reactions with iron and aluminum reduce the uptake of selenium by plants, as occurs in many parts of Europe (12). As a consequence, human selenium intake is too low and selenium deficiency diseases appear in the population of these areas. For that reason, selenium supplementation in animal feed and human food is necessary in these countries, although the question concerning which is the most suitable chemical species of selenium for supplemental use is still being debated.

The supplementation of selenium in dairy products is proposed (13, 14) as a good route by which to increase the human dietary selenium intakes, as cow's milk occupies a special place in the human diet because people drink it from childhood until old age. Therefore, the objective of this study was to investigate mechanisms for increasing the selenium

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Table 1. Operating Conditions for HGAAS<sup>a</sup>

		flow rate (mL	min <sup>-1</sup> )				
	pump 1	pu	mp 2				
step		carrier solution	reducing solution	time (s)	valve	read	function
1	10	9	5	10	fill		sampling
2	0	9	5	20	inject	*	Se hydride generation

<sup>a</sup> Wavelength, 196.0 nm; slit width, 0.7 nm; sample loop, 500  $\mu$ L; Ar flow rate, 125 mL min<sup>-1</sup>; quartz cell temperature, 850 °C; measurement mode, peak height; carrier solution, 4 M HCl; reducing solution, 0.2% NaBH<sub>4</sub> (m/V).

content in cow's milk samples and to study the element's distribution among the different components present in milk.

Hydride generation atomic absorption spectrometry (HG-AAS) was used to measure the levels of selenium in the various samples.

#### MATERIALS AND METHODS

**Apparatus.** An Ethos Plus microwave-assisted digestion system (Milestone, Sorisole, Italy) fitted with an internal temperature control was used to dissolve all of the project samples (maximum power, 1000 W; maximum temperature, 300 °C). A refrigerated ultracentrifuge L8 Beckman with an SW-40 rotor was used for the separation of whole cow's milk into its components. Measurements were carried out using a Zeeman 4100Z atomic absorption spectrometer equipped with an FIAS 400 system with a five-port flow injection valve, manifold, and separator gas—liquid used for hydride generation (all manufactured by Perkin-Elmer, Überlingen, Germany). A selenium electrodeless discharge lamp and an EDL system 2 power supply (also by Perkin-Elmer) were used throughout. **Table 1** details the HGAAS operating conditions.

**Reagents.** Ultrapure water, resistivity = 18 M $\Omega$  cm, was obtained from a Milli-Q water purification system (Millipore, Bedford, MA). Nitric acid 70.0%, hydrochloric acid 37%, hydrogen peroxide 35% v/v, and urea were all purchased from Panreac (Barcelona, Spain). Sodium tetrahydroborate was from Aldrich Chemical (Milwaukee, WI). Selenium stock solution 1.00 g L<sup>-1</sup> Se was prepared in 0.5 M nitric acid (Merck, Poole, Dorset, U.K.) (working standard solutions were prepared by suitable dilution of this stock solution). Certified reference materials NIST8435 (whole milk powder) from the National Institute of Standards and Technology (Gaithersburg, MD) and BCR281 (rye grass) from the Bureau of Reference Materials (Brussels, Belgium) were used to validate the analytical method and the instrument used in selenium determination.

Nutral Vacas A.R. (NUTRAL S.A., Madrid, Spain) was used to supplement forage with inorganic selenium as sodium selenite.

SEL-PLEX (Alltech Biotechnology, Barcelona, Spain) was used to supplement forage with organic selenium as selenized yeast (90% selenomethionine).

All glassware and plasticware were soaked for at least 48 h in a 10% v/v nitric acid solution and then rinsed several times with ultrapure water before use.

**Design of the Selenium Supplementation Studies.** Due to the seasonal variability of dietary selenium intakes, the feeding study protocol was carried out during a single time point in the year. However, in order that sufficient animals were available for the experiment, cows from three different dairy farms had to be used (rather than using one herd and performing the various feeding trials one after another, over a longer period). To minimize the variability between the animals used in the study, the farms were selected because they had cows of the same variety (Holstein Friesian), age, and lactation/sexual development. The farms were in a similar geographical location and of comparable size and operation mode. They all belonged to a cooperative, which required them to follow the same protocols with regard to feeding, milk collection, veterinary controls, etc. In addition to this, samples of forage were taken for analysis so that dietary inputs could also be taken into account when the data were interpreted.

 
 Table 2. Characteristics of the Dairy Farms Selected To Perform the Study and Total Mixed Ration (TMR) Composition

	control farm	farm B	farm C
no. of cows	60	40	40
milk production (L day <sup>-1</sup> )	1700	1160	1200
breed of cows	Holstein Friesian	Holstein Friesian	Holstein Friesian
TMR composition			
crude protein (% DM <sup>a</sup> )	16.8	16.9	17.1
neutral detergent fiber (% DM)	33.7	31.7	33.5
starch (% DM)	20.3	19.4	21.1
metabolizable energy concentration (Mcal kg <sup>-1</sup> )	1.7	1.7	1.7

<sup>a</sup> Dry matter.

 Table 3. Ingredient Composition of TMR and Selenium Concentration

 in Dietary Ingredients

	% DM			Se concn (µg	of Se kg-	<sup>1</sup> of DM)
ingredient	control farm	farm B	farm C	control farm	farm B	farm C
grass silage	23	33	31	65.0	49.8	53.6
maize silage	35	29	35	20.2	23.5	30.6
alfalfa		90	90		58.8	32.3
concentrate mixture	90	90	90	166.7	383.3	383.33
				DM i	ntake (kg)	
totals by weight	38.2	46.9	51.1	20.1	21.1	21.0

 Table 4.
 Selenium in TMR per Day in Each Farm as a Function of Selenium Supplementation

target supplementation	dietary Se co	ncn achieved
rate (µg of Se/day)	farm B	farm C
0.0 (natural Se levels in feed)	1891.4	1924.7
1800	3691.3	3914.7
2700	4591.4	4914.7
3600	5482.4	5914.7
4500	6382.2	6914.7

A total mix ration (TMR) was prepared to provide the same nutritional input (17% crude protein content referred to dry matter, 22–25% starch referred to dry matter, 1.7 Mcal kg<sup>-1</sup> of dry matter) to all of the farms. To prepare the TMR, the forage (a mixture of maize, grass, and alfalfa) from each farm was mixed with a concentrate mixture (cereals, soy, Se supplement) from the milk company in a mixing carriage prior to the TMR being deposited in the manger. **Table 2** details the dietary profile of the feeds and also provides additional information relating to the milk production on the three farms.

Study 1. Effect of Selenium Speciation on Levels of Enrichment Achieved in the Milk (Bioavailability Study). Table 3 presents the selenium content of the various components used to make the TMR to which the supplementation was added. Sufficient TMR was prepared to feed the cows for at least 40 days. Table 4 details the daily levels of additional selenium added to the animals' diets and the final concentration of selenium available to each animal.

On each farm, under each of the three feeding protocols, a sample of milk was taken at t = 0, that is, before supplementation, and then every 5 days for the next 40 days. During the period of supplementation, the milk samples were bulked into 1-day units (morning and evening); that is, sample 1 corresponds to the bulked milk sample collected on day 0 (without selenium supplementation), sample 2 represents the bulked milk collected on day 5, sample 3 represents the bulked milk collected on day 10, etc.

Study 2. Effect of Different Levels of Dietary Selenium Supplementation on the Concentration of Selenium in the Milk (Intervention Study). The dietary selenium intakes of cows on two farms were increased to four different levels, using the selenized yeast supplements. The selenium in selenized yeast is present as amino acids, the major component being Se-methionine. Two farms were used in the study so as to ensure that there was no between-farm difference in response to

Table 5. Aqueous Calibration and Standard Addition Slopes and Experimental (t<sub>exot</sub>) and Critical (t<sub>theor</sub>) t Values, 95% Confidence Level<sup>a</sup>

	standard addition <sub>WM</sub>		standard ac	standard addition <sub>WHM</sub>		standard addition <sub>CM</sub>	
curve	slope	t <sub>exptl</sub>	t <sub>theor</sub>	t <sub>exptl</sub>	t <sub>theor</sub>	t <sub>exptl</sub>	t <sub>theor</sub>
aqueous calibration	0.0202	40.538	2.09	40.02	2.09	43.023	2.09
standard addition <sub>WM</sub>	0.0139			1.92	2.09	0.867	4.303
standard addition <sub>WHM</sub>	0.0143	1.92	2.09			1.975	2.09
standard addition <sub>CM</sub>	0.0134	0.867	4.303	1.975	2.09		

a WM, whole milk; WHM, whey milk; CM, casein micelles.

the supplementation. The target levels for selenium supplementation were approximately 0, 1800, 2700, 3600, and 4500  $\mu$ g of Se day<sup>-1</sup>. **Table 4** presents the exact concentrations achieved and the natural concentration of selenium in the fodder supplied to each set of animals.

**Sampling Procedure for Milk Taken in Both Studies.** The cows were milked twice a day (morning and evening), and the milk was collected in refrigerated containers. Subsamples were taken from the containers into glass bottles; each bottle was labeled and kept at 4 °C during transportation to the laboratory. In the laboratory each sample was subsampled into several polypropylene tubes, which were kept frozen until required for analysis.

**Milk Sample Preparation.** Ultracentrifugation was used to obtain the three milk phases studied (15): fat, whey, and casein micelles. Milk samples (12 mL) were ultracentrifuged at 31000 rpm (160000g) for 60 min at 4 °C, with 1-min acceleration and 1-min deceleration times. The different components obtained were casein micelles and high molecular weight compounds as a precipitate, the whey in the middle of the ultracentrifuge tube, and an upper fat phase, and all of them were stored at -20 °C before analysis.

The sample pretreatment was carried out in three steps: In the first one, three replicate samples of whole milk, and of the different components of milk, were digested in a microwave oven. Each sample (2.5 mL for whole milk and whey milk and 0.9 g of casein micelles and fat) was weighed in the PTFE vessel, and 2.0 mL of nitric acid (70% v/v), 1.0 mL of hydrogen peroxide (35% v/v), and 2.5 mL of ultrapure water were added to perform the acid digestion process. The vessel was then heated to 200 °C for 10 min in the microwave oven. In the second step, after the vessel had been cooled to 50 °C, 1.4 mL of hydrochloric acid (37% v/v) was added, to reduce Se(VI) to Se(IV). Afterward, the vessel was heated again in the microwave oven to 130 °C for 10 min. In this stage, an important source of interference is nitrite (which results from the oxidative decomposition produced by nitric acid). The nitrite can form nitrogen oxides, which cause severe signal depression due to their oxidative potential against hydrogen selenide. To avoid this problem, a third step was included, in which the vessel was cooled to room temperature and 0.4 mL of urea solution (50% m/v) was added, to eliminate the excess of nitrogen oxides. Finally, the sample was transferred to a 25-mL volumetric flask and diluted to volume with ultrapure water.

Determination of Total Selenium in Whole Milk and the Separated Phases. The selenium determination in whole milk, milk whey, casein micelles, and fat phases was carried out by HGAAS after the described pretreatment of samples under the optimal conditions summarized in Table 1. The standard addition method was always used, due to the matrix effect that produces a decrease in the analytical signal.

**Forage Samples Pretreatment.** The homogenized and ground sample pretreatment was carried out following the same procedure used for milk pretreatment. The microwave-assisted acid digestion was performed with 0.5 g of sample, and only the volume of ultrapure water necessary in the first digestion stage was changed. Forage is a solid sample; therefore, 4 mL of ultrapure water was also added.

**Determination of Total Selenium in Forage.** To establish the natural Se level in forages (maize, grass, and alfalfa) used for the different farms, the Se content was determined using HGAAS. **Table 1** presents the instrumental conditions used.

### **RESULTS AND DISCUSSION**

Analytical Characteristics of the Method. Optimization of the Quantification Method, That Is, External Calibration Curve *versus Standard Addition.* Because of the complex nature of the milk matrix, a comparison of external calibration and standard addition quantification methods was performed. **Table 5** shows that statistical differences were observed between the external calibration and standard addition curves obtained with whole milk, whey milk, and casein micelles. However, no statistical difference was observed between the slopes of standard addition curves obtained for whole milk, whey milk, and casein micelles. Therefore, all data reported in this study, including that relating to the concentration of selenium in the forage samples, was obtained using the standard addition method.

Sensitivity. The detection limit is defined as 3SD/m (corrected for sample dilution and mass taken), where SD is the standard deviation measured in the procedural blank (n = 11) and m is the slope of the standard additions curve calculated for whole milk, whey milk, casein micelles, and the different forages. The limit of detection (LOD) values obtained for whole milk, whey milk, and casein micelles were 0.074, 0.065, and 0.075  $\mu$ g L<sup>-1</sup>, respectively. The LODs obtained for the forages were 4.1, 2.2, and 3.4  $\mu$ g g<sup>-1</sup> for grass, maize, and alfalfa, respectively.

*Precision.* The repeatability of the measurements, expressed as the relative standard deviation (RSD), was studied for the whole procedure for each different kind of sample. The values obtained were 4.4, 2.7, and 3.3% for the whole milk, whey milk, and casein micelles phase, respectively, and 4.9, 6.5, and 3.4% for grass, maize, and alfalfa, respectively.

Accuracy. NIST 8435 (whole milk powder) and BCR 281 (rye grass) with certified selenium contents of  $0.131 \pm 0.014$  and  $0.028 \pm 0.004 \ \mu g \ g^{-1}$ , respectively, were used to monitor the accuracy of the procedures used in this study. Five replicates of each material were analyzed, and the values obtained were  $0.129 \pm 0.005 \ \mu g \ g^{-1}$  for NIST 8435 and  $0.026 \pm 0.002 \ \mu g \ g^{-1}$  for BCR 281. There are no significant differences between the certified and obtained values (*t* test, confidence level of 95%). As there is no CRM for whey milk and case micelles, the analytical recovery was used to assess the accuracy of the data for these matrices. Mean values of 100.9 and 96.9% were obtained for the whey milk and case micelle samples, respectively.

**Concentration of Selenium in the Various Forage Samples. Table 3** presents the nonsupplemented selenium content of the forages, maize, grass, and alfalfa, from each farm. Four separate samples of each forage type were taken and analyzed in duplicate.

Study 1. Study of Selenium Supplementation as a Function of the Selenium Chemical Form: Bioavailability Study. To determine whether the procedures being used in the study were under statistical control, range and average control charts were drawn up. The significance level selected for the control charts used was ( $\mu \pm 3\sigma$ ) which, when 99.73% of the values lie within the limits defined (16), indicates that the process is under control. The range control chart data indicated that the system being studied was in a stationary state; that is, the variations observed

 Table 6. Concentration of Selenium in Milk Samples after

 Supplementation with Different Chemical Forms of Dietary Selenium<sup>a</sup>

	days of	S	Se concn ( $\mu$ g L <sup>-1</sup> )			
sample	supplementation	control farm	farm B	farm C		
0	0	$22.5\pm0.9$	$22.2 \pm 0.9$	$23.0\pm0.9$		
1	5	$22.9 \pm 0.5$	$23.6\pm0.4$	$30.0\pm0.7$		
2	10	$21.4 \pm 0.4$	$24.0 \pm 0.4$	$30.4 \pm 0.3$		
3	15	$22.1 \pm 0.6$	$23.3 \pm 0.7$	$32.2 \pm 0.3$		
4	20	$23.0 \pm 0.5$	$23.9 \pm 0.5$	$32.1 \pm 0.4$		
5	25	$22.4 \pm 0.7$	$24.1 \pm 0.5$	$31.7 \pm 0.3$		
6	30	$22.0 \pm 0.4$	$24.0 \pm 0.5$	$30.9 \pm 0.4$		
7	35	$22.1 \pm 0.4$	$23.0 \pm 0.5$	$30.9\pm0.5$		
8	40	$22.6\pm0.5$	$24.1\pm0.6$	$30.3\pm0.3$		

<sup>a</sup> Control farm, no supplementation; farm B, sodium selenite (0.3  $\mu$ g of Se g<sup>-1</sup>); farm C, selenized yeast (0.3  $\mu$ g of Se g<sup>-1</sup>). Results are expressed as mean ± SD (n = 12).



Figure 1. Time study of the changes in selenium concentration of milk from cows being fed similar amounts of selenium in different chemical forms.

were attributable to random factors (~4  $\mu$ g of Se/L). The average control chart data indicated that the process was under statistical control; that is, it did not change with time (variations within 2  $\mu$ g of Se/L).

**Table 6** presents the total selenium concentration measured in the eight samples collected from the three farms participating in study 1. **Figure 1** presents the changes in selenium concentrations achieved in the milk of the cows on the two different supplementation regimes. From the data, which have been corrected for the levels of selenium measured in the baseline (t = 0) sample for each group of cows, it can be seen that supplementation is much more effective when the selenium is in an organic chemical form. The plot also shows the speed at which dietary supplementation affects the milk selenium levels, that is, reaching a maximum within 3 days.

Selenium Distribution in Whey Milk, Casein Micelles, and Fat Phase. The selenium distribution in whey milk, casein micelles, and fat phase was studied for three milk samples

**Table 7.** Selenium Concentration in Milk Components (Milk Whey, Casein Micelles, and Fat Phase), Expressed as the Mean  $\pm$  Standard Deviation (n = 12)

		Se concn ( $\mu$ g L <sup>-1</sup> )		
	sample	whey milk	casein micelles	fat
control farm	1	$11.6\pm0.4$	$8.4 \pm 0.4$	$1.7\pm0.2$
	7	$11.8 \pm 0.3$	$8.5\pm0.5$	$1.8 \pm 0.1$
farm B (selenite)	1	$12.4 \pm 0.3$	$8.9\pm0.3$	$1.8 \pm 0.1$
	7	$12.3 \pm 0.4$	$9.0\pm0.4$	$1.9 \pm 0.1$
farm C (selenized	1	$14.1 \pm 0.3$	$11.4 \pm 0.4$	$2.5 \pm 0.1$
yeast)	7	$14.2\pm0.2$	$11.7\pm0.5$	$2.5\pm0.1$

obtained from the three farms. The samples were analyzed in triplicate using the standard addition method, and the selenium levels in whole milk, whey milk, casein micelles, and fat phase were determined. To check the values of the selenium determination in the different milk phases, a mass balance in six milk samples was performed. The selenium mass balance for the samples studied was between 95.5 and 100.8%. From the results obtained (Table 7), statistical analysis showed that significant differences [t test,  $\alpha = 0.05$  confidence level (17)] were observed in the selenium contents in the different milk components (whey milk, casein micelles, and fat) between samples from the farm where organic selenium supplementation was applied and samples from the other two farms. Moreover, no statistical differences were obtained in the selenium concentrations measured in the various components of the milk samples from the farms where the feed consisted of just forage or forage supplemented with sodium selenite.

The distribution of selenium in the various milk components, expressed as mean percentages, is shown in **Figure 2**. The highest selenium levels were found in the whey milk (with selenium percentages between 47.4 and 53.6%), whereas the lowest ones (between 7.3 and 9.3%) correspond to the fat phases. Although the milk supplemented with organic selenium contains a higher amount, the selenium distributions expressed as percentages were very similar in all cases.

These results show that it is possible to obtain seleniumenriched cow's milk without adding selenium to the milk, as is usual in the dairy industry, where inorganic selenium salts are directly added to the milk (18).

Study 2. Intervention Study. In this section of the study, the relationship between the amount of organic selenium (added as selenized yeast) and the increase of selenium incorporated into the milk was evaluated. As explained previously, to avoid the possible variations due to the differences between the farms (volume of milk, living conditions of cows, type of forages, etc.) and to know if these differences have any influence on this study, two farms were chosen and the feeding procedures were the same at both farms. Selenium concentration was determined in 10 cow's milk samples from each farm. The data for the two farms are presented in **Table 8** and show that the



**Figure 2.** Percentage distribution of selenium in the different components of cow's milk from the different farms: control farm, blank; farm B, sodium selenite (0.3  $\mu$ g of Se g<sup>-1</sup>); farm C, selenized yeast (0.3  $\mu$ g of Se g<sup>-1</sup>).

Table 8. Variation of the Selenium Concentration in Whole Milk Samples as a Function of the Amount of Selenium Supplemented to Forages<sup>a</sup>

suplementation	suplementation	Se concn ( $\mu$ g L <sup>-1</sup> )		
level ( $\mu$ g g <sup>-1</sup> )	time (weeks)	farm 1	farm 2	
0.0	1	$20.0\pm0.4$	$20.1\pm0.3$	
	2	$21.2 \pm 0.4$	$20.2 \pm 0.4$	
0.2	1	$27.2 \pm 0.4$	$28.7 \pm 0.3$	
	2	$26.6 \pm 0.4$	$28.3\pm0.4$	
0.3	1	$29.6 \pm 0.4$	$32.8 \pm 0.4$	
	2	$30.0\pm0.5$	$32.5\pm0.3$	
0.4	1	$31.6 \pm 0.5$	b	
	2	$32.5 \pm 0.3$	_	
0.5	1	$37.6 \pm 0.3$	$39.35\pm0.3$	
	2	$37.5\pm0.4$	$38.85 \pm 0.4$	

<sup>a</sup> Results are expressed as mean  $\pm$  standard deviation (n = 12). <sup>b</sup>-, milk sample not available.

 Table 9. Results from Multiple-Range Test Using

 Student–Newman–Kuels Method (95% Confidence Level) for Selenium

 Determination for Farms A and B

Se supple-	1	farm A		arm B
mentation		homogeneous		homogeneous
level ( $\mu$ g L $^{-1}$ )	mean	group	mean	group
0.0	20.05	Х	20.70	Х
0.2	27.45	Х	27.95	Х
0.3	29.80	Х	32.65	Х
0.4	32.05	Х	_a	_a
0.5	37.55	Х	39.17	Х
	contrast	difference	contrast	difference
	0-0.2	-7.4 <sup>b</sup>	0-0.2	-7.25 <sup>b</sup>
	0-0.3	-9.75 <sup>b</sup>	0-0.3	-11.95
	0-0.4	-12.0 <sup>b</sup>	_	_
	0-0.5	-17.5 <sup>b</sup>	0-0.5	-18.47 <sup>b</sup>
	0.2-0.3	$-2.35^{b}$	0.2-0.3	-4.7 <sup>b</sup>
	0.2-0.4	-4.6 <sup>b</sup>	_	-
	0.2-0.5	-10.1 <sup>b</sup>	0.2-0.5	-11.22 <sup>b</sup>
	0.3-0.4	-2.25 <sup>b</sup>	_	_
	0.3-0.5	-7.75 <sup>b</sup>	0.3-0.5	$-6.52^{b}$
	0.4–0.5	$-5.5^{b}$	-	-

<sup>a</sup> Values for level 0.4  $\mu$ g L<sup>-1</sup> on farm B are not available. <sup>b</sup> Statistically significant difference between the pair.

selenium content of milk increases with the level of selenium supplementation provided. The existence of a correlation between the selenium content in whole milk (WM) and the selenium level of supplementation in the feed was established by applying a linear regression: Z = a + bX (where Z is the selenium content in WM, X is the organic selenium added to the cow feed, and a and b are constants). Values of a and b as well as the correlation coefficient r were obtained by means of the software package Statgraphics Plus, version 5.0 (16). Thus, the correlation coefficients calculated were 0.982 for farm A and 0.994 for farm B. In addition, the correlation between different levels of selenium whole milk and the organic selenium supplemented is produced by the equations

farm 1: WM = 20.34 + 0.033X

farm 2: WM = 
$$20.48 + 0.038X$$

To prove the existence of a statistically significant difference among the levels of selenium in milk with variation in the selenium level of supplementation in the feed, a multiple-range test using the Student-Newman-Kuels method (95% confidence level) was performed (*16*). **Table 9** presents the results of the statistical analysis and shows that significant differences were obtained for selenium concentrations in milk related to the selenium level of supplementation in the feed.

**Conclusions.** Organic selenium, present as selenomethionine in a selenized yeast product, has been shown to be more bioavailable to cows than selenium in an inorganic form (selenite). This was proven by monitoring changes in the selenium content of milk produced by cows fed equivalent amounts of the two chemical forms of the element. Examination of the distribution of selenium between the various components in whole milk showed that supplementation using the selenized yeast did not affect the overall composition of the milk product, other than increasing the total amount of selenium present and the Se percentage in casein fraction.

The second part of the study showed that by supplementing the dietary selenium intakes of cows (using the selenized yeast product), the levels of selenium in the animals' milk can be elevated in a very controlled manner.

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