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Research Article

Selenium Concentrations in Serum and its Outputs in Milk and Urine from Grazing Jersey Cow Herds Found in Two Dairy Production Regions from Costa Rica

[1]Alejandro Saborío-Montero, [2]Margarita Alfaro-Cascante and [3]F. Granados-Chinchilla

[1]Escuela de Zootecnia, Universidad de Costa Rica, 11501-2060 Ciudad Universitaria Rodrigo Facio, San José, Costa Rica
[2]Unidad de Microbiología, Servicio Nacional de Salud Animal, 3-3006 Ministerio de Agricultura y Ganadería, Heredia, Costa Rica
[3]Centro de Investigación en Nutrición Animal (CINA), Universidad de Costa Rica, 11501-2060 Ciudad Universitaria Rodrigo, Facio San José, Costa Rica

Abstract

Objective: The study aimed to determine the concentration of selenium (Se) and glutathione peroxidase (GSH-Px) in serum and the Se concentration in milk and urine in grazing Jersey cows in two dairy producing areas of Costa Rica. **Methodology:** The study was conducted on commercial dairy herds in the highlands of Cartago (≈2250 m of altitude) and Zarcero (≈1750 m of altitude). Cartago cow herds were intensively grazing kikuyu grass (*Kikuyuocloa clandestina*) and Zarcero cow herds were grazing star grass (*Cynodon nlemfuensis*). Daily supplementation in both areas consisted of concentrate (16% CP, 1.81 Mcal NE_L) according to milk yield (1 kg concentrate: 3 kg of milk). Blood samples were taken from the coccygeal vessels, milk samples were collected individually during milking from the milk yield meter container and urine was obtained using rubbing stimulation technique. From Cartago area, a total of 102, 139 and 87 samples of blood, milk and urine respectively were collected and analyzed. From Zarcero region 66, 84 and 43 samples in the same order were collected and analyzed. **Results:** Atomic absorption spectrophotometry was used to determine Se concentration. A total of 85 samples from two farms in each region were tested to determine GSH-Px using a glutathione peroxidase activity colorimetric assay kit. The soil was tested in each farm using a soil auger to obtain 20 subsamples per sample, those subsamples were collected drilling the ground surface to a depth of 10 cm and then mixed to generate a composited sample which was analyzed for Se using atomic absorption spectrophotometry. Average serum, milk and urine Se concentration for cows from Cartago and Zarcero were 44.13 (SD = 27.68), 30.94 (SD = 20.13), 78.37 (SD = 60.14) μg Se L^{-1} and 19.19 (SD = 10.59), 21.82 (SD = 19.07), 14.72 (SD = 6.50) μg Se L^{-1}, respectively. The average GSH-Px concentration in serum was 73.74 and 33.82 for Cartago and Zarcero cows, respectively. **Conclusion:** High concentrations of selenium in urine in some of the farms and low concentrations of GSH-Px in serum in most of the cows could imply a poor utilization of this mineral, leading to deficiencies to meet metabolic requirements and therefore to associated economic losses.

Key words: Selenium, glutathione peroxidase, Jersey dairy cows, milk, blood, urine

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Corresponding Author: F. Granados-Chinchilla, Centro de Investigación en Nutrición Animal, Universidad de Costa Rica, 11501-2060 Ciudad Universitaria Rodrigo Facio, San José, Costa Rica Tel: +506 2511-2028 Fax: +506 2224-5527

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Selenium (Se) is considered a crucial element for both humans and animals, it has an intricate relationship with other key nutrients (e.g., vitamin E, lipids and sulfur). Like other minerals, Se must be obtained from dietary sources, as such, the mineral intake will depend on several factors including local availability of the mineral and the type of feedingstuffs[1,2] to name a couple. Hereafter, animals are subject to Se deficiencies which may affect their health severely[3-6]. This mineral scarcity is of particular importance in productive animals, such as dairy cows, in which adverse effects of Se deficiency include a decrease in weight gain[7,8], milk production, fertility[7,9], seminal quality[10] and immunological activity[11,12]. A selenium deficiency may also lead to disorders in the perinatal period altering milk quality in cows[13], placental retentions and the incidence of mastitis and metritis[6,14]. Insufficient Se level can even affect ruminant meat quality[15].

Although Se deficiency may occur in all animal species, ruminants seem to be the most likely to suffer it. Hence, this detrimental effects carry significant economic negative impacts. Likewise, though an unusual scenario, Se levels may also reach toxic concentrations if unchecked[16]. Hence, determination of the selenium status of livestock is paramount.

Selenium availability in gestating and lactating cows is critical, females transfer the element through the fetus (i.e., placental transfer) and to their progeny (colostrum and milk). In ruminants, this mineral transfer occurs even in deficient specimens; they sacrifice their condition to provide adequate levels to their offspring[4,17-19]. There is in fact, a documented reduction of Se in maternal plasma as gestation progress and the products increase in size and weight[17-19]. Considering the above, some dairy foods may result poor Se sources[20-22]. Although this situation has not been assessed in our country, some preliminary evaluations have been performed regarding Se concentrations in some relevant matrices such as soils[23,24] and forages[25].

Finally, in animals most Se is bound to protein, if limited an animal system will prioritize selenoenzyme synthesis for essential organs (brain, pituitary, thyroid and adrenal glands), under these conditions GSH-Px synthesis in blood is unessential[26-28]. Therefore, determination of GSH-Px is used as an indicator of the transformation of inorganic selenium to a bioactive species[26,29,30] and indirectly, the radical scavenging capability and oxidative stress of the animal (reducing cellular metabolism-related hydroperoxide activity)[31].

Considering the productive relevance of dairy cows, the determination of Se (in biological samples) as a diagnostic instrument in detecting mineral deficiencies[32] and at the same time to assess the mineral status of a particular region, the study aimed to evaluate Se concentrations in three different matrices (i.e., serum, milk and urine) of Jersey herds from two separate and relevant productive dairy regions from our country. Furthermore, analysis of GSH-Px in blood plasma was performed to measure, indirectly, the relative antioxidant capacity of the animal as related to Se.

MATERIALS AND METHODS

Study area and sampling: Two main dairy producing areas were selected for this study, Zarcero and Cartago (Fig. 1a, b).

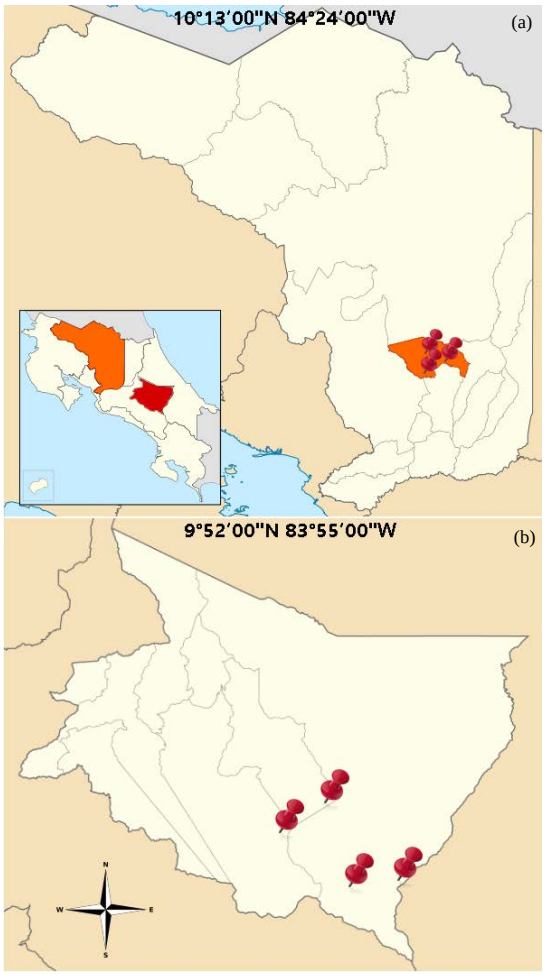

Fig. 1(a-b): Location map showing the survey sampling areas (a) Zarcero and (b) Cartago (regions identified in the orange and red filling, respectively)

A total of n = 3 and n = 4 commercial dairy farms were selected from each region, respectively, for sampling. Seven different Jersey dairy cow herds were considered for sampling during this survey, samples were collected from cows in various stages of lactation. A total of n = 66, n = 84, n = 43 and n = 102, n = 139, n = 87 samples of serum, milk and urine were recollected at each region, respectively on both accounts.

Blood: Samples were gathered from the tail (coccygeal vessels) of the cows, 5 mL of blood was drained using vacutainer 9 mL heparinized tubes (Becton Dickinson, Lith/Hep, New Jersey, USA). Samples were centrifuged soon after collection for the preparation of plasma which subsequently was stored frozen at -70°C until analyses were performed.

Milk: At each milking parlor, a representative aliquot of the milk from the milk yield meter container for each cow was placed in a Whirl-Pak® sterile sample bags (Nasco, Fort Atkinson, WI, USA) containing potassium dichromate as a preservative. Samples were retained at 4°C.

Urine: A sample of voided midstream urine was collected from each cow using the rubbing stimulation technique and a 4 oz sterile urine specimen container with screw-on lid cap (Pro advantage®, NDC, Tennessee, USA). The containers were stored at 4°C.

Selenium analysis: Method AOAC 986.15 was used during this survey. Briefly, 0.5 mL subsamples were treated with a 90:10 nitric acid and hydrogen peroxide mixture (both Merck suprapur; E. Merck, Darmstadt, Germany) followed by microwave digestion using a Berghof speedwave four (Harretstrasse, Eningen, Germany). Analyses were carried out by the flow injection metal hydride atomic absorption spectrophotometry (FI-MH-AAS) approach (Perkin-Elmer AAnalyst 800 atomic absorption spectrometer, Perkin-Elmer Corp., Norwalk, CT, USA). Hydride generation was performed using a 30 g L^{-1} $NaBH_4$ in 10 g L^{-1} NaOH and a 10 mL/100 mL HCl solution. Calibration curves for selenium were constructed from a selenium standard solution (SRM 3149, NIST, Gaithersburg, MD), Se concentrations ranged from 0.5-10 ng mL^{-1}. Calibration curves were constructed each time an analysis was performed, the resulting mean values for the curve were as follow:

$$y = [(1.37\pm0.43)x+1.61\pm0.51)]\times10^{-2}$$

The radiation source used was an electrodeless discharge lamp set at 45 mA, 0.7 L and 196 nm as for energy, spectral slit width and wavelength, respectively. The NIST SRM® 1549a (whole milk powder) was used as a quality control for Se when measurements were performed.

Serum Se-dependent glutathione peroxidase activity assay: A total of n = 85 (ca. n = 22 samples from two different farms from each sampling region) were tested to determine GSH-Px. In this case, the enzymatic activity analysis was performed using a glutathione peroxidase activity colorimetric assay kit (BioVision, Inc., Milpitas, CA, USA), as recommended by the manufacturer's protocol. Sample volume used during all tests was 1 µL of bovine serum. Briefly, GSH-Px reduces cumene hydroperoxide while oxidizing GSH to GSSG. The generated GSSG is reduced to GSH with consumption of NADPH, a decrease of NADPH is proportional to GSH-Px activity. All measurements were performed using a Synergy™ Biotek HT reader (λ = 340 nm) and the Gen 5™ software (BioTek Instruments Inc., Winooski, VT). A NADH standard curve was prepared using six individual standards; the resulting mean curve obtained during the assay was:

$$y = [(2.03\pm0.37)x+(3.74\pm0.42)]\times10^{-2}$$

Quantitative results for enzymatic activity were achieved in U mL^{-1}, which in turn were expressed as U g^{-1} of hemoglobin. An analytical GSH-Px enzyme standard available commercially was used as a control during the assay (G6137, Sigma-Aldrich, St., Louis, MO, USA).

Statistical analysis: Mann-Whitney U and Kruskal-Wallis tests were used to investigate significant differences between sampling regions and farms. Spearman's rho was used intending to assess association between the Se concentrations found in the three different types of samples collected and between those levels of Se found in serum and GSH-PX activity. A Wilcoxon sign-rank test was also applied to demonstrate a difference between the data set and the accepted minimum Se value considered apt for human consumption, optimum values recommended in bovine serum and to ascertain if selenium concentrations in soils were deficient. For all tests, values of $p<0.05$ were considered to be significant. Statistical tests and descriptive analysis of the results achieved were obtained from a truncated sample, those values considered as outliers were suppressed by trimming excluding values >3σ from the population's median.

All statistical tests were performed using IBM PAWS Statisticsl 22 (SPSS, Inc. Armonk, NY, USA).

RESULTS AND DISCUSSION

Physical-chemical properties of the soil of farms sampled: Volcanic soils are usually described to be lacking in Se and excess sulfur, which competes with the former for absorption, plants and animals that grow in these types of soil are usually reported to suffer from Se deficiencies[3,33]. The Se values in soils are correspondingly relevant since it is assumed that ruminants input is up to 18% of their dietary dry matter when grazing[34] and Se integration by the plant are influenced by physicochemical factors, such as soil's redox status, pH and microbiological activity[35] and even type of selenium available[36]. Soil type, texture, organic matter content and precipitation[5] determine selenium content, as well. Our survey shows that Se and S concentrations from soils collected within the sampling regions ranged from 78-345 μg kg^{-1} and 30-74 mg kg^{-1}, respectively (Table 1). Indeed, Se levels seem to be inadequate since concentrations of 500 μg Se kg^{-1} in soil or less and 0.1 ng kg^{-1} in plants are usually considered insufficient[3,33,37]. However, there is no apparent association between Se and S concentrations found in soil ($p>0.05$). Some reports have found a correlation between the Se levels in soils, plant and animal tissue and fluids[3,7,33,38]. Yet, even sufficient Se concentrations in soils may be hindered by other competing minerals such as Ca, Cu and As[39]. During our survey, we found concentrations of Ca and Cu as high as 15.7 cmol L^{-1} and 21 mg kg^{-1}, respectively (Table 1).

Selenium in serum or plasma: Our values are distributed between 44.13±19.07 and 19.19±10.59 μg L^{-1} (Table 2) for the regions of Cartago (1.95-141.42 μg L^{-1}) and Zarcero (1.95-74.40 μg L^{-1}), respectively. It was found significant differences in Se concentration in blood among the two regions analyzed and among herds examined ($p<0.001$). For example, differences were found between herds 1/2, 2/3, 2/4, 4/1 from Cartago and herds 5/6, 5/7 from the Zarcero region. Based on a relationship between Se amounts found in cows and mastitis, it has been proposed that Se should be present in bovine plasma and whole blood in at least 80 and 180 μg L^{-1}, respectively[40]. Hence, our data points toward a Se deficit status in the dairy cow. Notwithstanding, some individuals seem to possess satisfactory levels of the mineral (Table 2).

Selenium in milk: No clear guide has established an optimum Se concentration in milk for drinking[41]. However, it has been suggested that milk for consumption should at least reach values of 20 μg L^{-1} (values below 9 μg L^{-1} are considered deficient)[42], this concentration may supply up to 10% of the daily requirements of this mineral. There is evidence of a significant difference between the aforementioned recommended threshold and values of Se obtained from bovine milk sampled across both Cartago (1.22-90.13 μg L^{-1}) and Zarcero (1.22-95.60 μg L^{-1}) regions ($p<0.001$). Overall, sampling regions resulted in mean values of 30.94±20.13 and 21.82±19.07 μg L^{-1}, respectively (Table 2). Interestingly, no significant differences were found for milk concentrations among both sampling regions ($p = 0.32$). However, herd 2 exhibited the highest Se values and demonstrated to be significantly different compared to the rest of the groups ($p<0.05$). Individual differences were found between herds 2/4, 2/6, 2/7 and 4/5.

Detailed information about the contents of Se in cow milk (raw and skim milk) has been reported elsewhere. For example, Spain[43-48], India[49], Ireland[50], Kuwait[51], Sweden[52], Italy[53], United Kingdom[54], Croatia[55] and Netherlands[56]. However, the ranges reported vary immensely among reports, this may result from several factors such as animal breed[49,53], feeding[52,53], soil[53,57] and seasonal change[45]. The Se concentrations in European milk[58] have been described to be on average 11 μg L^{-1} whereas USA[59] values are reported to range between 7 and 87 μg L^{-1} and in Canada[60] mean values reach 28 μg L^{-1}.

Table 1: Mean values of soil chemical properties for the farms sampled (n = 21)

			cmol(+) L^{-1}					mg L^{-1}						
Location	pH	AS (%)	EA	Ca	Mg	K	ECEC	Fe	Mn	Cu	Zn	P	S	Se (μg kg^{-1})
Farm 1	6.1	0.7	0.17	15.7	6.6	1.4	23.9	358	26	17	32	173	74	98
Farm 2	5.5	8	0.44	3.7	1.1	0.5	5.8	165	15	9	1.1	5	35	78
Farm 3	6.2	1	0.17	11.4	3.6	1.0	16.2	343	19	8	34	73	57	144
Farm 4	6	0.9	0.18	12.8	5.4	1.3	19.7	245	10	10	25	46	32	345
Farm 5	5.2	3	0.26	5.5	1.9	0.5	8.2	192	28	21	7	10	30	270
Farm 6	5.9	2	0.17	7.4	2.0	1.3	11.0	153	32	15	5	6	31	215
Farm 7	5.8	0.7	0.23	9.4	3.4	1.0	14.1	243	21	13	17	52	29	166

ECEC: Effective cation exchange capacity, EA: Exchangeable acidity, AS: Aluminum saturation percentage

Table 2: Selenium concentrations found in the three different biological matrices sampled (n = 521)

	Concentration (μg Se L^{-1})			
Population/parameter	Mean±SD	Median	Max	Min
Serum				
Herd 1[ac]	24.91±18.49	23.40	86.63	1.95
Herd 2[b]	67.19±20.69	67.16	136.92	36.34
Herd 3[ca,cd]	36.25±23.59	36.90	78.18	7.86
Herd 4[dc]	41.44±25.89	37.44	141.42	2.08
Cartago area[u]	44.13±27.68	39.73	141.42	1.95
Herd 5[e]	14.49±13.95	11.10	74.40	3.20
Herd 6[fg]	20.85±5.74	18.94	34.75	12.70
Herd 7[gf]	23.44±6.71	21.75	37.53	10.70
Zarcero area[v]	19.19±10.59	17.44	74.40	1.95
Overall	34.10±25.55	26.06	141.42	1.95
Milk				
Herd 1[hi,hj ,hk]	32.61±21.16	27.90	90.13	7.45
Herd 2[ih,ij]	42.98±15.92	45.00	84.80	15.44
Herd 3[jk,jh,ji]	30.35±21.88	29.14	84.11	1.49
Herd 4[kh,kj]	22.35±15.97	22.40	49.33	1.22
Cartago area[x]	30.94±20.13	30.88	90.13	1.22
Herd 5[lm,ln]	26.68±26.96	16.00	95.60	1.80
Herd 6[ml,mn]	20.50±6.63	18.76	37.43	12.00
Herd 7[nl,nm]	15.41±5.04	15.19	30.70	8.43
Zarcero area[x]	21.82±19.07	16.65	95.60	1.22
Overall	27.51±20.23	21.77	95.60	1.22
Urine				
Herd 1[ño]	130.68±54.97	140.13	206.18	23.90
Herd 2[oñ]	139.62±36.25	135.14	206.80	86.00
Herd 3[pq]	37.35±18.52	33.99	98.10	12.53
Herd 4[qp]	28.35±12.13	22.90	49.00	8.90
Cartago area[y]	78.36±60.14	52.29	206.80	8.90
Herd 5[rs,rt]	9.79±7.63	8.85	34.50	0.10
Herd 6[sr,st]	17.16±4.33	15.85	24.78	10.55
Herd 7[tr,ts]	17.01±3.87	16.84	23.93	10.93
Zarcero area[z]	14.72±6.50	14.90	34.50	0.10
Overall	55.75±57.22	28.29	206.80	0.10

For each matrix, different superscripted letters, imply a significant difference between distinct herds, p<0.05

Table 3: Glutathione peroxidase activity in serum samples collected from 4 different dairy cow herds of two regions in Costa Rica (n = 85)

	Enzyme activity (U g^{-1} of hemoglobin)			
Parameters	Mean±SD	Median	Max	Min
Herd 2[a] (n = 22)	94.96±29.99	96.65	186.56	52.45
Herd 4[a] (n = 22)	55.64±28.63	55.32	121.73	6.77
Herd 6[b] (n = 22)	31.80±7.65	29.25	50.33	20.93
Herd 7[b] (n = 19)	34.82±9.00	32.70	54.04	18.27
Overall (n = 85)	55.09±33.43	43.07	186.56	6.77

Different superscripted letters imply a significant difference between herds, p<0.05

Selenium in urine: From the metabolic standpoint, urine excretion of the mineral is also intake-dependent[61]. In the case of dairy cows, an intake of 2 500 μg Se day^{-1}, results in losses by urine that round up to 500 μg Se[61] per day^{-1}. Urine values among herds and regions vary more drastically than those from serum or milk, mean values from Cartago (8.90-206.80 μg L^{-1}) and Zarcero (0.10-34.50 μg L^{-1}) regions are 130.37±196.44 and 14.72±6.58 μg L^{-1}, respectively (p<0.001). This variation is also reflected among herds where almost every farm (1/3, 1/4, 1/5, 1/6, 1/7, 2/3, 2/4, 2/5, 2/6, 2/7) show significant differences among them (p<0.05). Also, the distribution of Se concentrations found in blood and urine among the two regions differ significantly p<0.05. This fact points toward a differential herd management in each region and possibly to a dietary selenium of poor absorption, which is excreted swiftly. On the other hand, our data points toward a strong association between serum, milk and urine concentrations (p<0.001).

Selenoprotein activity: There is a high association between overall serum concentrations of Se and GSH-Px activities with a coefficient of determination of 0.941 (Cartago: R^2 = 0.91, Zarcero: R^2 = 0.95) and follows the equation:

$$y = 4.34x+1.31$$

This relationship can also be demonstrated by non-parametric tests (p<0.05), supporting other findings in which serum concentration and GSH-Px strongly correlates[62-65]. However, it is important to note that Avissar *et al.*[66] proved that positive correlations between Se concentration and GSH-Px activity in serum occur when Se levels are below requirement. This fact is endorsed by other findings[67,68]. Values of Se in human plasma ranging from 90.0[69]-98.7[70] μg of Se L^{-1} are assumed to be the requirement for full plasma GSH-Px expression, then the Se status of many study subjects would be considered derisory. Consistently, selenium deficient calves have been described with enzymatic activity levels of 9.82 U GSH-Px g^{-1} Hb, whereas animals reaching 100 U GSH-Px g^{-1} Hb are considered adequate[71]. In this regard, taken together with the rest of the evidence, this data suggests that Cartago area exhibit animals with a better Se profile, probably due to feeding practices (Table 2, 3).

Selenium in feedstuffs: Current regulation limit in-feed Se supplementation[72] to 300 μg kg^{-1}, it is assumed that this concentration can keep the animal with good mineral levels. It has been stated that an intake of 6 000 μg Se day^{-1} should be sufficient to maintain adequate concentrations[73]. Based on the levels in rations within the farms sampled, Se should be very well within these limits. Interestingly, the herd from the farm in which the feeding system is more structured resulted with significantly higher concentrations than the rest of the farms (Table 2, p>0.001). Most conventional feedingstuffs among farms included drinking water (1.78-8.45 μg Se L^{-1}), forage and silage (*Cynodon nlemfuensis* Vanderyst and *Lolium* spp., 21.21-61.21 μg Se kg^{-1}), compound feed

(21.15-424.73 μg Se kg^{-1}) and citrus pulp (135.46-235.67 μg Se kg^{-1}) and molasses (49.56-54.19 μg Se kg^{-1}). Other farms feed rations were comprised additionally by mineral supplement (54.19 mg Se kg^{-1}), distiller's dried grains with solubles (DDGS, 71.94 μg Se kg^{-1}), yeast (55.00 μg Se kg^{-1}), soybean and corn meal (587.73 μg Se kg^{-1}), hay (41.21 μg Se kg^{-1}) and animal fat (51.74 μg Se kg^{-1}). All values expressed on dry matter basis. As previously stated, different selenium feed sources influence profoundly in dairy cow selenium status. Recently, other researchers have described the impact of feedstuff and full rations on Se levels of final milk products[74,2]. From example, considering common inputs for each ration component (calculated in fresh biomass) and the total daily intake for a dairy cow[74], approximate Se contributions in decreasing order (based on feedingstuffs found in farm 2) are as follow: Mineral supplement 70.61% (from 80-120 g), compound feed 10.95% (4-6 kg), water 6.62% (50-60 L), forage 5.53% (20-22 kg), silage 3.82% (5 kg), citrus pulp 1.54 (0.5 kg) and DDGS 0.94% (0.5-1 kg) for an intake of ca. 16 μg day^{-1}. Previously, daily intakes per body weight have been reported: Ranging from <11 μg day^{-1} (China), 25 μg day^{-1} New Zealand, 79-104 μg day^{-1} and 113-220 in Canada[75]. Counterintuitively, a study revealed that selenium absorption was greater in sheep fed with a concentrate than in those fed a forage-based diet[76]. However, it should be stated that mineral levels in humans or animals are not governed solely by dairy ingestion of said nutrient but is related with the mineral bioavailability as well (i.e., distribution, association and chemical species)[77]. Bioavailability is of particular importance since absorption of selenium has been described to be much lower in ruminants than in other species due to the transformation of dietary selenium to insoluble forms in the rumen environment[78,79]. Hence, organic Se supplementation may be recommended[80].

CONCLUSION AND FUTURE RECOMMENDATION

Samples examined seemed, in general to follow agreeable values of Se set in other literature. Some extreme values in urine and relatively low in serum and milk may indicate specimens with overdosage of poor selenium sources. There is a strong association between the Se levels of the matrices assayed and in turn serum, Se concentrations associate with GSH-Px activity. However, average Se levels in milk are considered adequate for consumption. Other productive regions or relevant matrices should be regarded as is evident that each area is particular. Improved Se profiles are observed in farms with better feeding practices. Additional Se supplementation with bioavailable sources is recommended in the areas of study and we suggest the implementation of a vigilance program to consistently ensure mineral availability and acceptable levels in animals and food products alike.

SIGNIFICANT STATEMENTS

- Some relevant Costa Rican dairy-productive regions prove to be Se-deficient
- Se levels are adequate in farms where structured diets/rations are administered
- High values of Se in urine, low Se in blood and GSH-Px indicate a poor utilization of the mineral
- Se levels in serum, milk and urine associate strongly with each other
- Mean Se levels in milk are considered adequate for consumption

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