JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. 2003, 51, 4727–4731 4727

Monitoring of Feeds Selenium Status in a Southeast Region of Romania

MARIA SERDARU,*,† LUMIŅITA VLĂDESCU,‡ AND NICOLAE AVRAM†

PASTEUR Institute, 333 Calea Giulesti, 77826 Bucharest, Romania, and Department of Analytical Chemistry, University of Bucharest, 2-14 Av Regina Elisabeta, 70346 Bucharest, Romania

One hundred and eighty-five samples of feeds (hays, green plants, and concentrate feed) from households covering 41 localities in Dobrudja, a southeast region of Romania, were collected and analyzed for selenium (Se) content by spectrofluorometry with 2,3-diaminonaphthalene. Only 6.5% of the samples analyzed were, in terms of the feed Se content, considered appropriate (i.e., 0.15–0.30 ppm), within the normal range. The remaining 93.5% proved to be Se deficient; the results fell into the 0.001–0.150 ppm range. Consequently, the samples were divided into three deficiency groups based on the content recorded as follows: severe for 3.2% (Se below 0.01 ppm), critical for 84.9% (Se in the 0.01–0.1 ppm range), and marginal for 5.4% (Se in the 0.1–0.15 ppm range). Conclusively, the Dobrudja feeds may be said to be generally Se deficient, which requires prophylactic and therapeutic measures to correct animal selenium deficiency.

KEYWORDS: Selenium; fluorometric method; feeds

INTRODUCTION

Selenium (Se) is associated with a broad range of clinical conditions and metabolic activity in humans and animals (*1*). Many of these effects are caused by Se-containing proteins. The identification of more than 100 Se-containing proteins and the characterization of some of them (*2*) permitted the important role of Se in maintaining normal metabolism to be explained. Selenoproteins are involved in the antioxidant system, thyroid hormone metabolism, and redox regulation; they may have structural functions, which make them essential for optimal health. Clinical conditions, such as myopathies, are associated with Se deficiency and antioxidant cell system deterioration. Data have been gathered in recent years on the adverse effects of Se deficiency induced by the deterioration of other Se-dependent biochemical paths. These reactions are the basis for the decrease in immune response and increase in cancer incidence, known to be related to low Se content.

The importance Se holds for animals resides in its association with a number of pathological conditions: myopathies (lesions of the fibers or muscle groups such as the locomotor muscular system, respiratory muscular system, and cardiac muscle) (*3*), infertility (*4*–*7*), weight loss, alopecia, cataract, and liver and pancreatic conditions. Selenium deficiency may also generate an immunodeficiency syndrome (*8*–*10*).

As the feed is the primary Se source for animals, knowing the status of this trace element in the trophic soil–plant–animal chain has become necessary. Soil Se concentration depends on the content of the host rock. Selenium in the rock is released into the soil by erosion, of which a mere fraction is absorbed by the plants. This phenomenon depends on a number of factors including the soil type and pH, plant species, maturity status, performance, pasture management, and climate (*11*–*13*). Other factors involve the soil oxidoreduction conditions, humidity, and aeration degrees, that is, factors affecting the chemical Se forms and their bioavailability for plants. In acid, poorly aerated soils Se is in the form of selenide or metallic Se, forms that are quite insoluble and unavailable to plants. Selenium is available in acid, aerated soils, or those of a neutral pH where selenites form, as well as in alkaline aerated ones where it is in the form of selenates (*14*, *15*). The biological availability of Se to plants and subsequently to animals depends also on the presence or absence of other elements, which may enhance or inhibit its absorption (*15*). Plants have different abilities for absorbing Se. Because of these numerous factors, the Se content of the feed is difficult to predict.

On the basis of the fluorometric method of Se determination (*7*, *16*–*18*) this study aims at evaluating the Se status in feeds sampled in Dobrudja. This is an area in the southeast of Romania where clinical manifestations that may be attributed to the deficiency of Se were recorded. At present, there exists no systematic study regarding Se levels in Dobrudja feeds.

MATERIALS AND METHODS

Safety. The reagent 2,3-diaminonaphthalene (DAN) is very toxic and a possible carcinogen. Contact with the skin, eyes, and airways must be avoided. In case of accidental poisoning, medical advice must be sought immediately.

Method. Spectrofluorometry with DAN (*19*–*22*) was used to determine the Se content of the biological samples. The method consists

* To whom correspondence should be addressed. E-mail: pasteurserm@pcnet.ro.
† PASTEUR Institute.
‡ University of Bucharest.

10.1021/jf034113o CCC: $25.00
Published on Web 07/23/2003

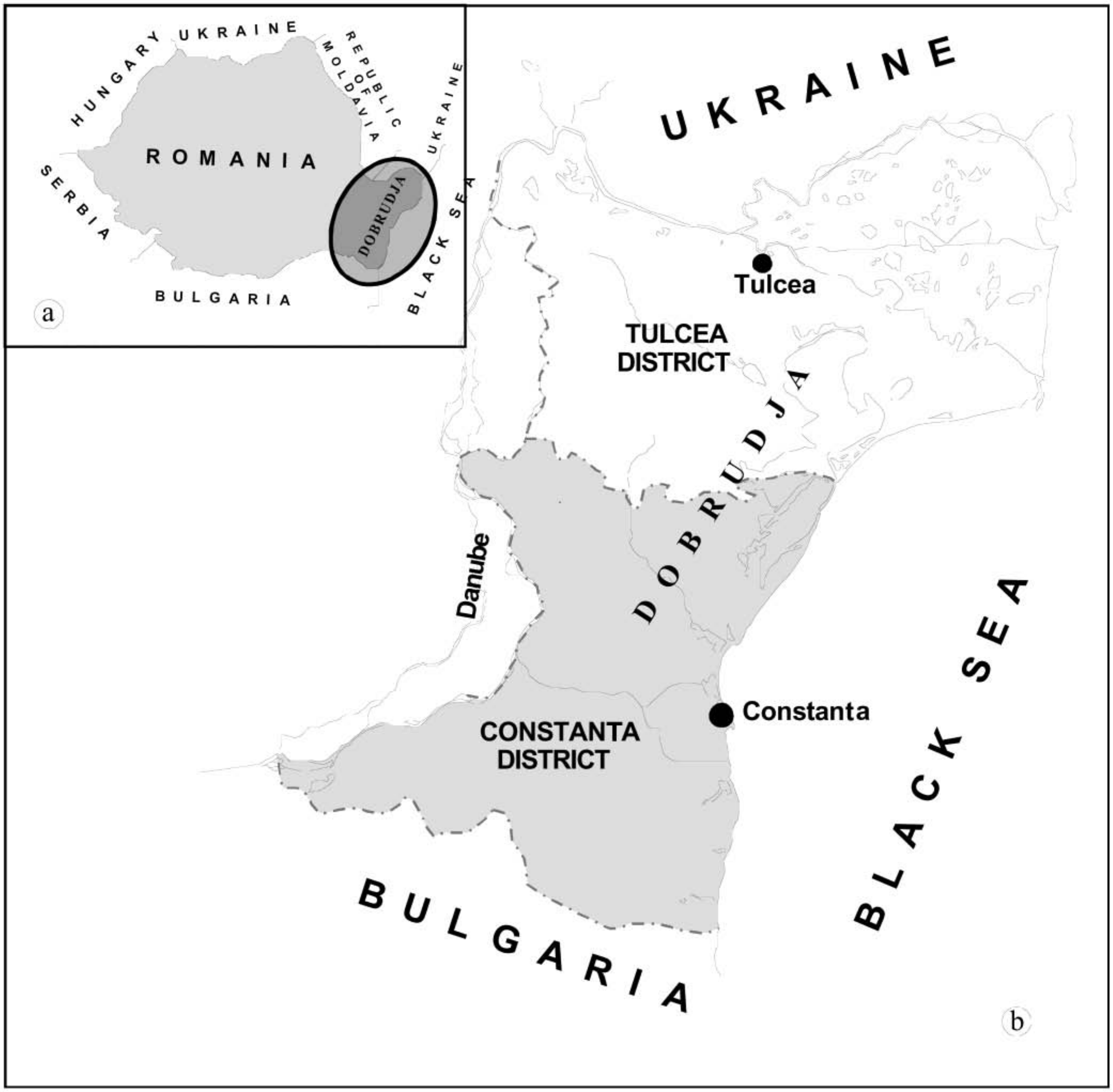

Figure 1. Location of sampling area. (a) Location of Dobrudja on Romania's map. (b) Dobrudja's administrative map.

of measuring the fluorescence of 4,5-benzopiazoselenol resulting from the reaction between Se and DAN. To this aim, the samples were mineralized in a strongly acidic medium, Se(VI) was then reduced to Se(IV)—the oxidation status reacting with DAN—in the presence of 6 M HCl. The interferences induced by Cu(II) and Fe(III) ions in the solution were eliminated by complexing the interferers with complexon III (suppurr).

Reagents. Only reagents of analytical purity were used as follows: (i) the mixture of acids (Merck) for the mineralization process was prepared as follows: HNO_3 (65%):$HClO_4$ (70%):H_2SO_4 (97%) = 9:3:1; (ii) hydrochloric acid (Merck) at dilutions 6, 0.6, and 0.1 M; (iii) ammonia (Merck) 25% and diluted as a 2.5% solution; (iv) complexing agent (CHK): 2.69 mM disodium salt of ethylenediaminetetraacetic acid, 28.78 mM hydroxyammoniumchloride, and 50 mg/L bromocresol purple; (v) the reagent DAN (Sigma) as a 0.01% solution in 0.1 M HCl (because it is photosensitive, the reagent was prepared in semidarkness at a maximum of 60 °C); (vi) cyclohexane (Merck) of chromatographic purity; and (vii) 1 mg of Se/mL of standard stock Se solution (titrisol Merck).

Samples. One hundred and eighty-five samples were collected as follows: 86 from the Constanţa district and 99 from the Tulcea district. From among these, 48 were green plants (green wheat, green alfalfa, and green barley), 33 were fibrous feeds (mixed hay, grass hay, *Lolium perenne* hay, alfalfa hay, rye hay, and clover hay), and 104 were concentrate feeds (sunflower seeds, wheat grain, barley grain, oat grain, corn grain, etc.). About 200 g of each was sampled.

The samples were collected from households in 41 localities of Dobrudja: 16 were located in the Constanţa district, and 25 were in the Tulcea district (**Figure 1**).

Calibration Curve Plotting. The standard stock Se solution was used to prepare an intermediate standard solution (0.05 mg Se/mL) from which working standard solutions (0, 0.01, 0.02, 0.06, 0.10, 0.20, 0.30, 0.40, 0.50, and 1.00 μg Se/mL) were prepared by successive dilutions with double-distilled water. Volumes of exactly 0.1 mL were measured from each working standard solution and then added to 0.3 mL of the acid mixture and subjected to mineralization (225−230 °C, 10 min). The solutions obtained were each acidulated with 0.1 mL of 6 M HCl for the reduction of Se(VI) to Se(IV) by keeping them at 140

Table 1. Green Plant Se (ppm) Concentration

	Constanţa district				Tulcea district				total Dobrudja			
sample	*n*	$\bar{x}$	SD	range	*n*	$\bar{x}$	SD	range	*n*	$\bar{x}$	SD	range
green wheat	9	0.013	0.007	0.003–0.021	12	0.031	0.014	0.006–0.059	21	0.003	0.014	0.003–0.059
green alfalfa	8	0.043	0.024	0.013–0.084	9	0.113	0.075	0.016–0.221	17	0.080	0.066	0.013–0.221
green barley	5	0.022	0.013	0.001–0.036	5	0.042	0.017	0.021–0.066	10	0.031	0.018	0.001–0.066
total green plant	22	0.026	0.021	0.001–0.084	26	0.060	0.059	0.006–0.229	48	0.045	0.048	0.001–0.22

Table 2. Fiber Fodder Se Concentration (ppm)

	Constanţa district				Tulcea district				total Dobrudja			
sample	*n*	$\bar{x}$	SD	range	*n*	$\bar{x}$	SD	range	*n*	$\bar{x}$	SD	range
mixed fodder	2	0.037	0.001	0.036–0.037	4	0.060	0.023	0.042–0.092	6	0.046	0.016	0.034–0.057
grass hay	2	0.046	0.016	0.034–0.057	1	0.046		0.046	3	0.046	0.012	0.034–0.057
Lolium perenne hay	2	0.029	0.006	0.025–0.033					2	0.029	0.006	0.025–0.033
alfalfa hay	12	0.054	0.028	0.014–0.096	7	0.059	0.024	0.024–0.089	19	0.056	0.026	0.014–0.096
rye hay	1	0.029		0.029					1	0.029		0.029
clover hay					2	0.025	0	0.025	2	0.025	0	0.025
total fibers	19	0.048	0.024	0.014–0.096	14	0.053	0.023	0.024–0.092	33	0.050	0.024	0.014–0.096

°C for 2 min. One milliliter of CHK was then added to each solution, and the pH was adjusted to 2.0−2.4 with 0.1 M HCl and 2.5% ammonia. Se(IV) was then complexed with 2 mL of DAN at 60 °C for 30 min. The resulting fluorescent compound (4,5-benzopiazoselenol) was extracted with 2 mL of cyclohexane and fluorometrically dosed, $\lambda_{excitement} = 366$ nm and $\lambda_{emission} = 520$ nm. The fluorescence of each solution was measured against the control sample containing all of the reagents except Se. The calibration curve—$y = ax + b$ (where $a = 0.67$, $b = 0.002$)—is linear on the 0.01−1.00 ppm concentration range.

Sample Digestion. The samples—each about 0.1 g homogenized and weighed at a ±0.0002 g precision—were first dried at 60 °C for 2 h and then mineralized at 225−230 °C with 0.3 mL of the acid mixture until a colorless liquid resulted. The solutions obtained were each acidulated with 0.1 mL of 6 M HCl and kept at 140 °C for 2 min for the reduction of Se(VI) to Se(IV).

Se Content Measurement. The solutions resulting after sample digestion were each treated with 1 mL of the complexing agent and adjusted to pH 2.0−2.4 by means of 0.1 M HCl and of 2.5% ammonia. The Se(IV) complexing with 2 mL of DAN at 60 °C for 30 min led to the formation of 4,5-benzopiazoselenol, a fluorescent compound that was, after the extraction with 2 mL of cyclohexane, fluorometrically dosed ($\lambda_{excitement} = 366$ nm, $\lambda_{emission} = 520$ nm).

Equipment. A Spekol 11 spectrometer (Carl Zeiss Jena) equipped with fluorometry annexes was used.

RESULTS AND DISCUSSION

The course of several pathological conditions specific for Se deficiency led to a systematic study of the Se status of the feeds. To this aim, 185 samples of various feeds were collected as follows: green plants (48 samples), hays (33 samples), and concentrate feed (104 samples). The samples originated from various households in Dobrudja.

Dobrudja is a geographical area in southeast Romania located between the Danube and the Black Sea. It is guarded by low, eroded mountains surrounded by large plateaus in the north, by the Danube delta in the northeast, and by a tableland turned into an erosion plain in the south. The climate is, except for the sea shore, continental and dry. Dobrudja is divided into two districts, Tulcea and Constanţa, located in the north and south, respectively (**Figure 1**).

The test samples were from both the Tulcea district (99) and the Constanţa district (86). The Se content was determined by the previously described fluorometric method. The results are presented in **Tables 1−4**. Each group of feeds was characterized based on the number of samples (*n*), the mean value ($\bar{x}$), standard deviation (SD), variation coefficient (CV), and variation range (range).

Table 1 presents the results of green fodder Se content determination. Of the 48 samples of green feed under analysis, only three alfalfa samples were within the 0.15−0.30 ppm range indicated in the literature as the adequate feed Se content (*23*). Thus, the green fodder samples from the Constanţa district proved to be 100% Se deficient, and those from the Tulcea district were 88.5% deficient. The richer in Se fodder proved to be the green alfalfa samples that recorded the highest values for both districts.

Another test category was fibrous fodder. The results (**Table 2**) characterize this fodder group as totally Se deficient. None of the samples investigated reached the acceptable minimum threshold of 0.15 ppm. However, alfalfa and grass hay, along with the mixed fodder, recorded a higher Se content than the other hays under analysis.

As for the concentrate feed (cereals) group, the results show that of the 45 Constanţa district samples only one exceeded the 0.15 ppm threshold, while of the 59 Tulcea district samples seven did (**Table 3**). Thus, the former and the latter are 97.78 and 88.14% Se deficient, respectively. On comparing the results obtained with these two groups, the Tulcea district samples were found to be richer in Se than the Constanţa district ones, with only one exception: the oat grain. The Se richest and poorest feed assortments were the sunflower and grain oat, respectively.

Figure 2 compares the mean values for all three feed categories under analysis, i.e., the green plants, fibrous feeds, and concentrates. On the whole, the samples collected in the Tulcea district had a higher Se concentration as compared with those collected in the Constanţa district.

Table 4 presents the results of Se determination in the three feeds groups: the concentrate feeds have a slightly higher Se content than the other two groups.

Of the samples under analysis, those feed assortments allowing an adequate statistic analysis ($n > 10$) were selected, and the mean Se concentration was determined. The results showed the samples of sunflower seeds, alfalfa (either green or as hay), and wheat grain to be slightly richer in Se, much below the minimal threshold of 0.15 ppm (**Figure 3**).

For the Se content of all of the feeds analyzed, the range was 0.001−0.364 ppm. The ratio between the highest and the

Table 3. Compound Feed Se Concentration (ppm)

	Constanţa district				Tulcea district				total Dobrudja			
sample	*n*	$\bar{x}$	SD	range	*n*	$\bar{x}$	SD	range	*n*	$\bar{x}$	SD	range
sunflower seeds	7	0.050	0.024	0.033–0.100	12	0.148	0.065	0.083–0.235	19	0.112	0.072	0.033–0.235
wheat grain	11	0.042	0.014	0.024–0.065	14	0.072	0.073	0.028–0.261	25	0.059	0.056	0.024–0.261
barley grain	8	0.039	0.013	0.020–0.054	9	0.057	0.035	0.026–0.133	17	0.048	0.028	0.020–0.133
oat grain	2	0.062	0.001	0.061–0.062	2	0.048	0.001	0.047–0.049	4	0.055	0.008	0.047–0.062
corn grain	15	0.044	0.038	0.013–0.156	19	0.061	0.078	0.006–0.364	34	0.053	0.063	0.006–0.364
mixed concentrates	2	0.021	0.014	0.011–0.031	3	0.091	0.091	0.023–0.194	5	0.053	0.075	0.011–0.194
total concentrates	45	0.043	0.026	0.011–0.156	59	0.083	0.075	0.006–0.364	104	0.065	0.061	0.006–0.364

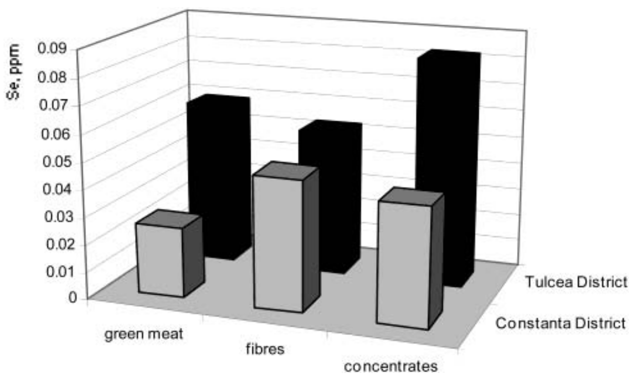

Figure 2. Se ($\bar{x}$, ppm) distribution by feed categories in the two districts analyzed.

Table 4. Se (ppm) Distribution by Feed Groups of Dobrudja

feeds	*n*	$\bar{x}$	SD	CV (%)	range
green feed	48	0.045	0.048	108.46	0.001–0.221
fibers	33	0.050	0.024	47.07	0.014–0.096
concentrates	104	0.065	0.061	94.32	0.006–0.364
total feeds	185	0.057	0.054	94.20	0.001–0.364

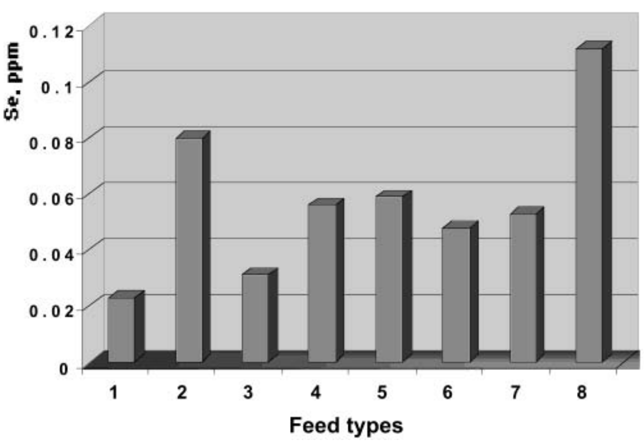

Figure 3. Se ($\bar{x}$) distribution in various feeds types of Dobrudja. 1, Green wheat ($n = 21$); 2, green alfalfa ($n = 17$); 3, green barley ($n = 10$); 4, alfalfa hay ($n = 19$); 5, wheat grain ($n = 25$); 6, barley grain ($n = 17$); 7, corn grain ($n = 34$); 8, sunflower seeds ($n = 19$).

lowest Se concentration determined was 364, which demonstrates the high variation of the individual values. Of the 185 samples investigated, only 12 (cca 6.5%) recorded an appropriate Se content (within the 0.15−0.30 ppm range). The other samples proved to be Se deficient being grouped—depending on the Se content—into three deficiency groups (**Figure 4**): severe deficiency (Se content below 0.01 ppm, cca 3.2% samples), critical deficiency (Se content within 0.01−0.10 ppm, 84.9%

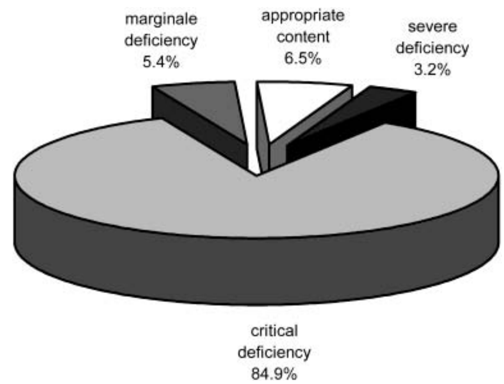

Figure 4. Distribution—%, by deficiency levels—of Se deficiency recorded with the feeds collected in Dobrudja.

samples), and marginal deficiency (Se content within 0.10−0.15 ppm, 5.4% samples).

The data obtained confirm that the Dobrudja area of Romania may be considered to be Se deficient. The Se feed deficiency recorded compares to the literature data characterizing other areas in Europe—Norway, Finland, and Denmark as well (*15*).

The results of this study reveal that feeds originating from Dobrudja have a Se content that is both quite variable and, with a few exceptions, below the requirements for animal feed. This characterizes the feeds sampled from the two districts, Constanţa and Tulcea, although in the latter case the values were higher for all of the feed groups. The highest mean values were those for the green alfalfa and sunflower, while the lowest ones were with the green wheat.

Depending on the Se content recorded, the samples were arranged into three deficiency groups: severe, critical, and marginal deficiency including 3.2, 84.9, and 5.4% of the samples, respectively; the remainder (6.5%) were within the normal range. Because Se deficiency was evident with the great majority of the samples investigated (93.5%), the improvement of animal health status and performance through intensive supplementation programs is a necessity.

ABBREVIATIONS USED

DAN, 2,3-diaminonaphthalene.

LITERATURE CITED

(1) Arthur, J. R.; Beckett, G. J. New metabolic roles for selenium. *Proc. Nutr. Soc.* **1994**, *53*, 615−624.
(2) Arthur, J. R.; Bermano, G.; Mitchell, J. H.; Hesketh, J. E. Regulation of selenoprotein gene expression and thyroid hormone metabolism. *Biochem. Soc. Trans.* **1996**, *24*, 384−388.
(3) Radostits, O. M.; Bloo, D. C.; Gay, C.C. Veterinary Medicine. Diseases cause by deficiencies of mineral nutrients. *Veterinary Medicine*, 8th ed.; Baillière Tindall: London, 1994; pp 1408−1425.

(4) Arthur, J. R. Nonglutathione peroxidase functions of selenium. In *Biotechnology in the Feed Industry, Proceedings of the 13th Annual Symposium*; Lyons, T. P., Jacques, K. A., Eds.; Nottingam University Press: Nottingham, U.K., 1997; pp 143−154.
(5) Surai, P. F. Organic selenium: Benefits to animals and humans, a biochemist's view. In *Biotechnology in the Feed Industry, Proceedings of the 16th Annual Symposium*; Lyons, T. P., Jacques, K. A., Eds.; Nottingam Press: Nottingham, U.K., 2000; pp 205−260.
(6) Behne, D.; Weiler, H.; Kyriakopoulos, A. Effects of selenium deficiency on testicular morphology and function in rats. *J. Reprod. Fertil*. **1996**, *106*, 291−297.
(7) Hansen, J. C.; Deguchi, Y. Selenium and fertility in animals and man − A review. *Acta Vet. Scand.* **1996**, *37*, 19−30.
(8) Larsen, H. J. Influence of selenium on antibody production in sheep. *Res. Vet. Sci*. **1998**, *45*, 4−10.
(9) Levander, O. A.; Ager, A. L.; Beck, M. A. Contrasting and interacting nutritional determinants of host resistance to parasitic and viral infections. *Proc. Nutr. Soc.* **1995**, *54*, 475−487.
(10) Turner, R. J.; Finch, J. M. Selenium and the immune response. *Proc. Nutr. Soc.* **1991**, *50*, 275−285.
(11) Reid, R. L.; Horvath, D. J. Soil chemistry and mineral problems in farm livestock. A review. *Anim. Feed Sci. Technol*. **1980**, *5*, 95.
(12) McDowell, L. R. In *Nutrition of Grazing Ruminants in Warm Climates*; Academic Press: New York, 1985.
(13) McDowell, L. R.; Conrad, J. H.; Hembry, F. G. In *Minerals for Grazing Ruminants in Tropical Regions*, 2nd ed.; University of Florida: Gainesville, 1993.
(14) Mayland, H. F. *Selenium Responsive Diseases in Food Animals*; Proceedings of the Western States Veterinary Conference, February 18; Schering Corporation: Kenilworth, NY, 1986.
(15) Oldfield, J. E. *Selenium World Atlas*; Selenium Tellurium Development Association: Grimbergen, Belgium, 2002.
(16) Alfthan, G. Micro-method for determination of selenium in tissues and biological fluids by single-test tube fluorimetry. *Anal. Chim. Acta* **1984**, *165*, 187−194.
(17) Brown, M. W.; Watkinson, J. H. An automated fluorimetric method for the determination of nanogram quantities of selenium. *Anal. Chim. Acta* **1977**, *89*, 9−35.
(18) Van Saun, R. J.; Herdt, T. H.; Stowe, H. D. Maternal and fetal selenium concentrations and their interrelationships in dairy cattle. *J. Nutr.* **1989**, *119*, 1128−1137.
(19) Izbash, O. A.; Karpov, Yu. A.; Pletenev, T. V.; Shiryaeva, O. A. Determination of selenium for biomonitoring. (A review). *Zavod. Lab*. **1992**, *58* (9), 3−9.
(20) Sheehan, T. M. T.; Gao, M. Simplified fluorimetric assay of total selenium in plasma and urine. *Clin. Chem.* **1990**, *36* (12), 2124−2126.
(21) Alaejos, M. S.; Romero, C. D. Analysis of Selenium in Body Fluids; A Review. *Chem. Rev*. **1995**, *95*, 232−234.
(22) Serdaru, M.; Vlădescu, L. Study on the Fe(III) and Cu(II) ions interference in determining Se(IV) by 2,3-diamninonaphthalene fluorimetry. *Rev. Chim. (Bucharest)* **2002**, *52*, 582−586.
(23) NRC. Nutrient requirements of domestic animals (No. 5). *Nutrient Requirements of Beef Cattle*, 7th ed.; National Academy of Sciences, National Research Council: Washington, DC, 1996.

Received for review February 4, 2003. Revised manuscript received March 21, 2003. Accepted March 27, 2003. This work was supported by ORIZONT 2000, the National Research and Development and Invention Program financed by the Ministry of Education and Research of Romania.

JF034113O