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Blood Selenium and Vitamin E Levels in Heifers: Regional and Seasonal Differences in Northern Cyprus

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SS and HK conceived and designed the study. SS collected the samples, performed the experimental work and wrote the article. SS and HK analyzed the data.

Key words
Selenium, Vitamin E, Feed, Heifer, Northern Cyprus.

ABSTRACT

The aim of this study was to establish the current state of blood selenium and vitamin E levels in heifers, together with roughage selenium levels in eight livestock regions of Northern Cyprus from two different seasons. We also evaluated whether these concentrations were adequate. Heifer blood, feed samples including concentrate feed or roughage were analysed. It was found that there are significant seasonal differences for each region and regional differences in each season were due to different feeding conditions. Heifer blood selenium and plasma vitamin E levels were generally found within adequate ranges in all regions for both seasons. We suggest that feed supplements containing selenium should be added for the Iskele region during the winter months. For the Morphou region we advise that feed supplements should be added during summer due to low levels of selenium found in roughages.

INTRODUCTION

In the past, Selenium (Se) toxicity attracted much attention, however in subsequent years, through extensive research, metabolic diseases were found to be associated with a deficiency of Se, such as white muscle disease in lambs and calves (Whanger *et al*., 1977), exudative diathesis (Hassan, 1986) and pancreatic degeneration in poultry (Thompson and Scott, 1969). It's also known that Se has also play critical role in maintenance of pregnancy, uterine health, fertility (Arechiga *et al*., 1998; Kamada *et al*., 2014), meat and milk yields and immune functions (Hall *et al*., 2014).

The main source of Se is through the daily ingestion of food by living organisms and dietary intakes have been associated with a range of biochemical functions and diseases in animals. Se plays a particular role as selenocystein at active site of proteins (Pedrero and Madrid, 2009). Some well-known selenoproteins, in which Se is an essential trace element for their activity, are glutathione peroxidase, thioredoxin reductase, iodothyronine deiodinase, selenoprotein P, selenoprotein W, selenoprotein N (Beckett and Arthur, 2005; Lescure *et al*., 1999; Richardson, 2005; Rotruck *et al*., 1973; Schomburg *et al*., 2004).

Research has shown that most areas of the world are poor of the intake of Se and metabolic diseases are seen as endemic in those areas due to Se deficiency. In the United States approximately two-thirds of dairy cows are reared in the areas where soils are poor in selenium and arid regions of Australia, the north-eastern China, South China, north of Democratic People's Republic of Korea, Nepal, Tibet, Central Africa, Pakistan, the Democratic Republic of Congo are known to be Se deficient (Hogan *et al*., 1993; Khan *et al*. 2005, 2012; Poppenga *et al*., 2012; Wichtel *et al*., 2004).

Se and vitamin E are functionally closely linked as the part of antioxidative system. Vitamin E is an essential dietary nutrient for the healthy development of animals. Deficiency in vitamin E is mostly observed in juvenile animals, and when combined with Se deficiency, more serious health problems can arise (Castellini *et al*., 2007). Vitamin E has physiological and biochemical roles including the regulation of protein activation, immunity, gene expression, protection of selenoproteins, preventing free radical damage as an antioxidant scavenger and maintaining the amphipathic balance of cell membranes (Wang and Quinn, 1999).

Establishing the situation of Se and vitamin E levels, both regionally and nationally is extremely important in order to determine the scarcity or abundance of these molecules, which can be directly linked to animal health. Such studies would be beneficial in order to determine

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regional profiles and therefore enable individuals or governments to take the necessary precautions. Considering the importance of Se and vitamin E in livestock health, the main goal of the current study was to determine blood Se, feed Se and plasma vitamin E levels in heifers from eight livestock regions in Northern Cyprus from two different seasons. An additional benefit of this study would allow for comparisons to be made between both regionally and seasonally and therefore enable assessments to be made on the necessities of feed supplements.

MATERIALS AND METHODS

Animals and sample collection

This study was approved by Animal Experiments Ethic Committee of Ankara University, Ankara, Turkey. In Northern Cyprus, there are eight livestock regions according to the Veterinary Department which is affiliated to the Ministry of Agriculture and Natural Resources. These regions are Nicosia, Gecitkale, Vadili, Famagusta, Iskele, Ziyamet, Morphou and Kyrenia (Fig. 1). The main type of livestock is cattle breeding and herds only consisted of Holstein dairy cows.

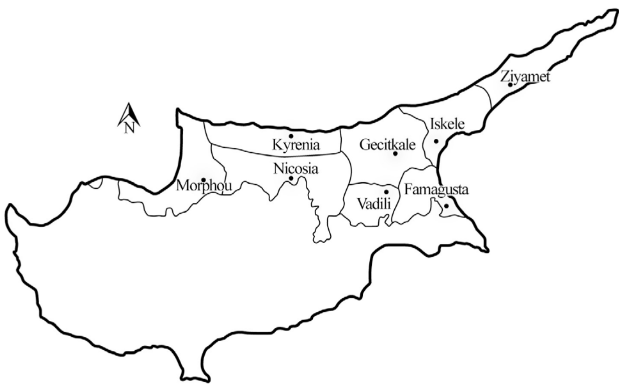

Fig. 1. Map of livestock regions of Northern Cyprus (Veterinary Department, Ministry of Agriculture and Natural Resources, Northern Cyprus).

Heifers were chosen from cattle population in order to investigate the status of Se and vitamin E in Northern Cyprus. Thus, some confounding factors can be eliminated; such as period of lactation, parity, dry periods and gestation which may affect the evaluation of results. Blood samples were collected from heifers during two different periods. These were during the arid period of July to August 2008 and the rainy period of December 2008 to January 2009. In both periods, samples were collected from at least 30 animals for each region to be statistically evaluated. Twenty-nine dairy farms located in rural areas belonging to each region were randomly selected to monitor Se and Vitamin E status and the distribution of these farms were as following: 4 farms in Nicosia (n=32), 4 farms in Gecitkale (n=37), 3 farms in Vadili (n=32), 2 farms in Famagusta (n=32), 5 farms in Iskele (n=31), 6 farms in Ziyamet (n=32), 2 farms in Morphou (n=33), 3 farms in Kyrenia (n=31). Dairy herd farms were generally below 100-head and were family businesses. Blood samples were individually collected from jugular vein of clinically healthy heifers using 9 ml of two different heparinized blood tubes and cooled immediately. One of the heparinized blood tubes was used for Se analysis as whole blood and the other was used to obtain plasma for vitamin E analysis by centrifugation at 1500 x *g* for 10 min for each sample.

It is also well known that Se content in roughages based on soil Se content shows seasonal fluctuations. Seasonal changes like acidity, annual rainfall, moisture, availability of pasture, fertiliser application can affect soil structure. Thus, accumulation and intake of minerals may vary (Khan *et al*., 2006). This is the reason that feed samples, including both roughages and concentrated feeds, were also collected to evaluate Se content in parallel with the blood Se levels in each season. All feed materials used for animal nutrition in each farm were collected for Se analysis and no knowledge of the feeding regime was known at the time of sampling. Particular attention was paid to feed materials taken from the front of the animals and storages. Locked freezer bags were used to collect feed samples. Feed samples varied between farms due to the fact that each farm used different roughage types and feed concentrates. Feed types included bales (wheat, barley and corn), clover, wheat, corn, cottonseed meal, barley seed and own-made commercial concentrate feeds. After blood and feed collections during each period, all samples were transferred to the laboratory of the Department of Biochemistry, Faculty of Veterinary Medicine, Ankara University, where they were stored in a freezer at -80ºC until analysed.

Se analysis

To determine whole blood and feed Se concentrations, a fluorometric method was used, previously described by Koh and Benson (1983). According to this method, sample digestion is required to destroy any organic matter and sample reduction to convert the Se into the Se (IV) oxidation state. An acid mix which contains $HClO_4$ and HNO_3 was used to digest both blood and feed samples. The reaction of Se (IV) with 2, 3-diaminonaphthalene (DAN) to form a fluorescent Se-DAN heterocyclic compound is the basis of the fluorometric method for Se determination. Piazselenol, the reagent formed as a result of the reaction with DAN, has greater fluorescence sensitivity. The Piazselenol complex was then extracted and the fluorescence intensity was measured at an excitation wavelength of 313 nm and an emission of 430 nm using spectrofluorometer. The calibration curve obtained from the data of sodium selenite solution was treated using the same process which was applied to the aforementioned samples.

Vitamin E analysis

Vitamin E analysis of plasma samples was performed using liquid chromatography–tandem mass spectrometry with atmospheric pressure chemical ionization (LC-APCI-MS-MS) as previously described by Andreoli *et al*. (2004). Vitamin E analysis was performed in a private laboratory called Ankalab, Ankara. An ethanol/ethyl acetate mixture was used for both protein precipitation and antioxidants solubilisation. Deproteinized samples were injected into the LC-APCI-MS-MS system. Vitamins were separated on a C-8 column using a methanol/dichloromethane mixture and ionized using a positive-ion mode; detection was performed in the selected-reaction monitoring mode. For control of assay, two level of α-tocopherol quality control serums were used.

Statistical analysis

Regional and seasonal changes of Se and vitamin E were analysed using two-way analysis of variance (Two-way ANOVA) (Kutsal *et al*., 1990). Significance levels were considered as $P<0.05$ and $P<0.001$.

RESULTS

Whole blood Se levels

In order to interpret the selenium blood levels, the criteria's laid out by Dargatz and Ross (1996), were adopted. This criteria enables the classification of individual cattle and the ranges between 0 and 50 ng/ml is severely deficient, 51-80 ng/ml is marginally deficient, 81–160 ng/ml is adequate and 161 ng/ml or more is highly adequate. Blood Se levels for heifers were determined to be at adequate levels in all regions during both seasons (Table I). In summer, Se levels in Gecitkale region were found to have the lowest values and were significantly different when compared to Nicosia, Famagusta, Iskele and Ziyamet ($P<0.001$). In the comparison of the results during the winter, both Morphou and Kyrenia regions were significantly different from the other regions ($P<0.001$). Famagusta region was found to have the lowest values and close to being marginally deficient. When both summer and winter seasons for each region was compared, significant differences were found in Nicosia ($P<0.05$), Famagusta, Iskele and Ziyamet ($P<0.001$).

Feed Se levels

Feed samples collected from the eight regions were grouped as roughages and feed concentrate according to their structures. According to the National Research Council (NRC) (2001) and (2005), roughage Se concentrations of ≤ 100 *n*g/mg are associated with clinical Se deficient livestock. In all regions, and across both seasons, Se levels of roughages showed significant differences (Table I). Additionally, during winter in Iskele and in the summer in Morphou, Se levels were found below and on the borderline, respectively, according to NRC (2001) and (2005), levels.

Se levels of feed concentrate in all regions and for both seasons were found to be adequate according to daily requirements (0.3 mg/kg NRC, 2001). Furthermore, the region of Famagusta was found to show a significant difference when it was compared to other regions, with the exception of Vadili and Iskele regions ($P<0.05$) during winter (Table I).

Plasma vitamin E levels

According to Herdt and Stowe (1991), Radostits et al. (2007) and Weiss (1998), plasma vitamin E levels ≥ 2 mg/*l* is considered as adequate. Accordingly, all regions for both seasons, plasma vitamin E levels were found to be adequate (Table II). Some regions and seasons, showed significant differences in plasma vitamin E levels.

DISCUSSION

The deficiency and excessiveness of Se and vitamin E can cause many problems in livestock animals, therefore many studies have been taken as examples from different regions of world in order to shape this research, which is the first of its types on heifers in Northern Cyprus. There have been studies in the gulf of Morphou, Famagusta and the Kyrenia coastline which have investigated heavy metal pollution and also examined the agricultural geography of the island. Nevertheless, the content of these studies does not include Se or vitamin E and animal health as their primary target (Abi, 2006; Atimtay and Saricicek, 2001; Dinc et al., 2000; Ercal, 2007; Erdem et al., 2001).

According to unpublished data, it is known that some metabolic disorders such as white muscle disease in lambs, retained placenta, infertility are seen in Northern Cyprus and Se and vitamin E levels may play a role in these disorders. However, it is mandatory to verify the related roles of these two molecules in these disorders. To overcome this question, the initial first step was to investigate the regional and seasonal differences of Se and vitamin E.

Table I.- Se levels of whole blood (ng/ml), roughages (ng/mg) and feed concentrates (ng/mg) of heifers according to regions and seasons.

Regions	Summer				Winter				P
	n	Mean±SEM	Min	Max	n	Mean±SEM	Min	Max	
Whole blood of heifers									
Nicosia	32	227.13±31.68 $^{a.B}$	79	790	31	137.53±12.75 $^{b.AB}$	40	367	*
Gecitkale	37	154.44±14.61 ^{A}	53	527	32	153.77±11.31 ^{B}	40	316	-
Vadili	32	189.68±21.76 $^{A.B}$	26	650	31	162.00±15.42 ^{B}	20	275	-
Famagusta	32	241.79±21.76 $^{a.B}$	121	632	31	98.54±11.94 $^{b.A}$	26	277	***
İskele	31	339.14±23.26 $^{a.C}$	92	606	31	148.27±11.08 $^{b.B}$	40	263	***
Ziyamet	32	327.89±28.08 $^{a.C}$	178	751	30	160.88±22.12 $^{b.B}$	20	474	***
Morphou	33	217.18±24.37 ^{AB}	17	538	31	262.77±17.71 ^{C}	113	462	-
Kyrenia	31	210.14±11.36 ^{AB}	92	369	31	245.49±20.82 ^{C}	105	632	-
Roughages									
Nicosia	7	202.80± 29.42 ^{BC}	40.00	284.40	7	189.13±39.09	52.67	335.27	-
Gecitkale	7	132.11±31.18 ^{AB}	40.00	252.80	7	212.48±49.20	123.32	430.91	-
Vadili	7	313.83±29.82 $^{a.D}$	205.40	442.40	7	155.47±45.03 ^{b}	63.20	393.07	*
Famagusta	...	…	...	...	...	...	...	...	
İskele	7	237.77±39.23 $^{a.C.D}$	80.00	354.54	7	60.08±2.01 ^{b}	52.27	63.20	***
Ziyamet	7	162.81±20.49 ^{ABC}	79.00	221.20	7	196.18±89.22	52.67	758.40	-
Morphou	7	97.14±17.14 ^{A}	40.00	160.00	7	134.37±28.95	63.20	277.00	-
Kyrenia	7	157.37±25.26 ^{ABC}	40.00	221.20	7	127.90±42.97	31.60	316.00	-
Feed concentrate									
Nicosia	7	463.52±58.60	197.50	677.14	7	394.43±43.88 ^{A}	263.33	526.67	-
Gecitkale	7	580.40±113.33	395.00	1241.43	7	392.60±38.30 ^{A}	201.09	430.91	-
Vadili	7	596.53±185.05	225.71	1580.00	7	472.03±74.97 ^{AB}	143.64	718.81	-
Famagusta	7	710.03±229.06	126.40	1692.86	7	710.03±229.06 ^{B}	126.40	1692.86	-
İskele	7	595.72±150.03	79.00	1241.43	7	458.76±75.89 ^{AB}	189.60	790.00	-
Ziyamet	7	313.55±43.48	197.50	451.43	7	217.25±22.67 ^{A}	131.67	263.33	-
Morphou	7	410.39±7.25	395.00	430.91	7	357.03±80.24 ^{A}	143.64	632.00	-
Kyrenia	7	322.44±68.79	197.50	677.14	7	228.72±21.89 ^{A}	158.00	316.00	-

-, P>0.05; *, P<0.05; ***, P<0.001; $^{a, b}$, Different letters in the same row are statistically significant; $^{A, B, C, D}$, Different letters in the same column are statistically significant; ¶, Roughages were not obtained because ration contains only concentrated feed.

Table II.- Plasma vitamin E levels (mg/l) of heifers according to regions and seasons.

Regions	Summer				Winter				P
	n	Mean ± SEM	Min	Max	n	Mean ± SEM	Min	Max	
Nicosia	32	6.05±0.22 ^{D}	4.5	10.8	31	6.78±0.42 ^{D}	4.24	11.7	-
Gecitkale	37	6.33±0.24 ^{D}	3.84	9.86	32	5.84±0.37 ^{B}	4.08	16	-
Vadili	32	5.43±0.14 ^{C}	4.06	7.58	31	5.72±0.09 ^{B}	4.84	7.21	-
Famagusta	32	4.69±0.10 ^{B}	3.89	6.94	31	4.84±0.06 ^{A}	4.2	5.69	-
Iskele	31	4.38±0.09 $^{a.AB}$	3.37	5.35	31	4.99±0.12 $^{b.A}$	3.86	6.58	***
Ziyamet	32	4.48±0.11 $^{a.AB}$	3.11	5.58	30	5.07±0.10 $^{b.A}$	4.07	6.26	***
Morphou	33	4.14±0.09 $^{a.AB}$	3.34	5.47	31	4.38±0.07 $^{b.A}$	3.81	5.12	*
Kyrenia	31	4.79±0.13 ^{B}	2.79	6.08	31	4.96±0.26 ^{A}	0.29	7.04	-

-, P>0.05; *, P<0.05; ***, P<0.001; $^{a, b}$, Different letters in the same row are statistically significant; $^{A, B, C, D}$, Different letters in the same column are statistically significant.

Norway has Se and vitamin E deficiency problems and the data obtained from the Norwegian Cattle Health Recording System play an important role in the development of livestock (Schomburg *et al*., 2004). The animal registration system in Northern Cyprus is not a well-developed and heifers were chosen in this study in order to eliminate confounding factors such as milk yield, number of births, nutrition and lactation period.

Similar studies from different parts of world have previously reported Se and vitamin E levels with regional and seasonal fluctuations in different animal classes (Ahmad *et al*., 2013; Gadberry *et al*., 2003; Khan *et al*., 2012; Kommisrud *et al*., 2005; McComb *et al*., 2010; Siversten *et al*., 2005; Yildiz *et al*., 2006). Certain factors, such as heavy metals can affect Se and vitamin E metabolism at the absorption level. The blood levels of both molecules in Northern Cyprus were shown to be in similar to values found in the Norway (Siversten *et al*., 2005). When the results were evaluated in terms of regional and seasonal differences, blood Se levels have shown more interesting distributions, when compared to that of vitamin E.

Differences in concentrated feed and roughages in animal rations were observed in every sampled herd. The Se levels of roughages in Iskele during winter and in Morphou during the summer were determined below 100 ng/mg (Table I). However, this was not reflected in the blood samples from the animals of these regions. This may have been a result of high Se levels in the feed concentrate when the main diet ratio was concentrated feed. On the other hand, in the Morphou and Iskele regions, mineral levels in plants can be affected by climate, mineral composition, soil pH and plant type. In the Morphou region there are closed copper mines which could possibly affect the selenium cycle during the summer months, due to the fact that selenium is extracted as a by-product of copper mining and this can lead to Se deficient soils (Haug *et al*., 2007).

In Norway, high blood Se levels of Norwegian Red Heifers and dry period cows have been shown to be a causative factor in disorders such as mastitis, ovarian cysts and anoestrous, it is therefore stated that feed additives should be used (Kommisrud *et al*., 2005). It is essential to obtain the information such as animal health, disease incidences and productivity in order to reveal Se and vitamin E status and its association with such disorders. Therefore, a reliable assessment was not possible in Northern Cyprus, despite the levels of both molecules being within normal ranges.

Even if forage additives containing Se and vitamin E or roughages containing adequate amounts are used, this does not mean an animal will take its dietary daily requirement. Individually, animals may not intake nutritional requirement due to certain reasons such as diet ratio, stress, pregnancy, lactation period and climatic conditions. Although, forages containing adequate amount of both molecules are provided, deficiencies are seen in some regions of world and little differences are shown between places where forage additives are used or not used (Dargatz and Ross, 1996; Pehrson *et al*., 1997). In the present study, feed samples varied between each farm because each farm used different roughages and concentrate feed types. Farms in Famagusta did not use any roughages in the rations for both seasons. Se levels in Famagusta region were shown to have great differences between summer and winter (Table I). One of the sampled farms prepared its own concentrate feed and especially used a high ratio of cottonseed meal in the diet. The ingredients of feedstuff used in diets caused significant differences in some regions and seasons. Additionally, the vast majority of breeders obtain their concentrate feed from a manufacturer and these manufacturers add vitamins to their concentrate feed. Perhaps this is why there were little differences between regions during the two seasons.

There is a correlation between blood Se levels and animal feed type. Animals fed with only local grass can show inadequate levels, this may be caused by extensive farming in regions which contain low levels of soil selenium (Fenimore *et al*., 1983). When the Iskele region is considered, there is a significant difference in blood Se levels across both seasons and this difference seems to be in parallel with the difference between roughage. This may have resulted from the use of high ratio of roughages in the diet. This could show a correlative effect between Se levels in animals and Se levels in different types of forages.

On the other hand, a previous study conducted in Turkey reported that there was no relationship between Se levels in animals and forage materials. The reason for this result was thought to be based on variable Se levels in forages, the amount that animals ingest and also the additives used (Yildiz *et al*., 2006). When results of this study were evaluated, similar situations arose, for instance, in the Nicosia region Se blood levels for both seasons were found to be statistically different (Table I) in spite of the fact that there was no significant difference in concentrate feed and roughage levels (Table I). This situation may be explained by amount of consumption of forage materials and also presence of other elements like sulphur which prevents the uptake of Se. In case of vitamin E, significant differences were also determined between some regions and seasons, despite finding adequate blood levels (Table II). These levels may be a result of differences found in the various plants used, since these plants are the main source of vitamin E.

CONCLUSIONS

Se and vitamin E levels of heifers were found within normal ranges for animal health in all regions during two seasons. This study was an initial step to investigate Se and vitamin E profile in Northern Cyprus. However, it was found that inadequate levels of Se in roughages are used in some regions and seasons, it is therefore important to prepare a balanced diet containing both concentrate feed and roughages, especially in the regions of Iskele, Morphou and Famagusta. Supplements can also be provided in these regions during both seasons.

Another goal of this study was to determine ranges of blood Se and plasma vitamin E levels in heifers for both seasons and use them to make a database for heifers in Northern Cyprus. It has also diagnostic significance to determine ranges and is used to identify nutritional disorders. The blood Se levels obtained in heifers in the present study were ranged from 154.44 to 339.14 ng/ml in summer and 137.53-262.77 ng/ml in winter. The ranges of plasma vitamin E levels obtained in heifers in the present study were 4.14-6.33 mg/l in summer and 4.38-6.78 mg/l in winter.

As the main source of Se is soil, detailed soil analysis is thought to be essential for the full profile of Se in Northern Cyprus. The reason for this is cattle breeding is being performed intensively and the main source of nutrition for animals is concentrate feed whose ingredients are mostly imported and which also contains supplements. Therefore, this kind of research should also be applied to sheep and goats which are bred intensively or semi-intensively. This information could also be used not only for Northern Cyprus, but also for all islands and Middle Eastern countries with similar geographical and climatic conditions.

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This article is part of study was summarized from Ph.D. thesis of SS. This work was also presented as orally presentation in the 1st International Congress on Advances in Veterinary Sciences and Technics on (25-29 August 2016, at Sarajevo, Bosnia and Herzegovina.

Conflict of interest statement

We declare that we have no conflict of interest.

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