

Study of Selenium Concentration in Serum and Milk of Sheep Flocks (Lori-Bakhtiari Strain), Iran

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Abstract: This experiment was performed to assess selenium (Se) content of blood and milk of Lori-Bakhtiari sheep flocks of Chahar Mahal and Bakhtiari, Iran. Blood and milk samples have taken from six different regions of mentioned province at May and June. From each region, two herds and ten dairy sheeps per flocks selected. The average amount of Se in serum samples were 0.0233, 0.0348, 0.0232, 0.0208, 0.0284 and 0.0379 $\mu\text{g g}^{-1}$ DM and in milk samples were 0.0045, 0.0057, 0.0045, 0.0049, 0.0057 and 0.0379 $\mu\text{g g}^{-1}$ DM. Comparison of means did showed the significant different between Se concentration of serum and milk of six sheep herds of mentioned state regions ($P < 0.05$). Regarded the presented results in this study, it is concluded that Se concentration of blood and milk of Lori-Bakhtiari sheep herds were lower than standards concentrations.

Key words: Selenium concentration • Diet • Serum • Milk • Local sheep

INTRODUCTION

Plant species differ in the amounts and concentrations of Se which they potentially absorb. Thus, they are classified as excluders, passive absorbers, or accumulators, if they usually absorb less than 50, 50 to 100, or more than 100 $\mu\text{g g}^{-1}$, respectively [1]. Often, the plants are simply referred to as non-accumulators or accumulators. The actual Se uptake is controlled not only by the plant species, but also by the activity of the various Se forms in the soil and the amount of soil water present. Most soils contain no more than 0.1 $\text{Se } \mu\text{g g}^{-1}$, but those derived from the Cretaceous shale may contain 1 to 2 $\mu\text{g g}^{-1}$ and some may have values as high as 500 $\mu\text{g g}^{-1}$ [2]. Soil Se exists in several chemical forms that differ widely in their solubility and availability to plants [3]. These forms include selenide (Se^{2-}), elemental Se (Se^0), selenite (Se^{+4}), selenate (Se^{+6}) and organic forms. Most of the plant-available soil Se occurs as selenate and selenite. Plant uptake from these 2 sources has been investigated extensively and results have been summarized by Mikkelsen *et al.* [4].

Accumulator plants retain the absorbed Se as water-soluble selenite and non-protein organic forms and non-accumulator plants metabolize much of the Se

into protein bound selenomethionine or selenocystine [5]. Organic Se may be volatilized from shoots, actively excreted from roots of growing plants, or mineralized from decaying vegetation [6]. Thus it is demonstrated that organic Se forms have been found in soils.

Selenium has been shown to be an essential micro-nutrient for mammals, birds and several bacteria [7]. Various selenium containing amino acids occur in nature and play important physiological roles especially in grazing sheep. Selenium after absorb from plants roots transferred to tissues and milk accompanying with plasma protein. More than 80% of protein-bound Se is selenocysteine. The regulation and synthesis of these proteins and its behavior in the different organs and tissues are highly dependent on selenium supply. If selenium is limited, the system gives priority to the central organs (brain, pituitary, thyroid, adrenals) for the synthesis of selenoenzymes; in these conditions the blood GSH-PX is the last priority [8, 9,10].

It is well-known that there is a significant link between selenium rate of blood and milk. Because Se is capable to passage easier from cell membranes of mammary gland and added to milk volume. Hence Se concentration of milk is highly depend on it's content in diet [11]. Se concentration of milk changed one week after

alteration of diet Se content. Supplemented sheep, increased Se levels in the allantoic fluid, milk and colostrums and their lambs had better weight gain in the first two weeks of life [12]. Newborns obtain Se through the colostrums and milk, thus Se availability of the mothers is critical in lactation gains [13, 14].

Selenium is a necessary trace mineral for body. The role of selenium in animal and human health and diseases has been discussed in detail in several recent reviews, with the main conclusion being that Se deficiency is recognized as a global problem which needs solving urgently. The relationship between diet and human health has received substantial attention in the last few years, with the realization that unbalanced diets can cause serious health-related problems. In addition, those animals that produce human food, such as milk (dairy), meat and eggs not only need Se but also have to transfer it to human diets. Deficiency in the animal diets is associated with some dangerous diseases such as myopathy and or nutritional muscular dystrophy (white muscle disease, WMD) in young lambs, calves and goats, lameness, decrease of productivity, retained placenta [15, 16, 17], also increased infertility and immune dysfunction. The effect of Se on female fertility and litter size has given contradictory results; some authors have reported positive effects with supplementation [18-22].

Se deficiency affects blood levels of IgG and T cell function and this determines a higher prevalence and severity of present diseases in animal populations [23]. Because of decrease of the antigen processing and antigen presentation, the humoral response can be limited [24-26]. The use of Se as an immune system stimulant has a positive impact on the immune response and quality of the colostrums [27].

In humans, Se deficiency is associated with a compromised immune system and increased susceptibility to various diseases, including arthritis, cancer, cardiovascular disease, cataracts, cholecystitis, cystic fibrosis, diabetes, immunodeficiency, lymphoblastic anaemia, macular degeneration, muscular dystrophy, stroke and some others [28]. The most compelling evidence exists in relation to the cancer-protective effects of Se [29, 30].

Therefore, aim of the presented study, was investigation of selenium (Se) content of serum, milk and diet of sheep flocks (Lori-Bakhtiari strain) in different regions of Chahar Mahal and Bakhtiari state, Iran which supposed to be on the redline.

MATERIALS AND METHODS

Diets, Husbandry and Sampling: The current study focused on six regions of Lori-Bakhtiari state because of the existence of the same geographic conditions which respectively are Shahrekord (region 1, R1), Ardel (R2), Farsan (R3), Lordegan (R4), Brujen (R5) and Koohrang (R6) cities at May and June (Figure 1). Because industrial farms of sheep rearing have established in some of selected regions, therefore study performed on local grazing dairy animals from each area of mentioned state.

Status and necessary data such as plant and diet type of pasture region, sheep phenotype and multiplicity, rearing management system type, mineral and vitamin premix and drugs possible used in herds (above 100 heads multiplicity), were collected prior to beginning of recording and study. Twenty herds of dairy sheep were selected from six mentioned regions (10 heads dairy sheep per herd) and numbered as ear punched and daily monitored. The diet, blood and milk samples were taken at two time period, May and July during one day. To collect diet samples, predominant variety of plants in region pastureland specified prior to sampling. Then, amount of 1.5 kg plant removed from different parts of grassy area where sheep grazed, (from multiple-harvest and six samples) collected for each region from the distance of about 1 cm above the collar was taken by a small sickle and samples were placed in special paper envelopes and paper doors were closed after the registration papers of all regional profile, including name and pasture, sheep unit name and the collecting time, has been moved to the proper environment. Sample clean up after their extra Brushwood, were weighing and their fresh weight was noted. Then, fresh plant samples at room temperature in degrees shade conditions were dry and the amount of dry forage each sample was calculated.

A numbers of 10 sheep for sampling from milk and blood were selected. Blood samples, from the left jugular vein of sheep was collected into the vacuum tube without anticoagulant substance. Prior to blood sampling, the area has been cleaned and the vein has been located. This has done by using a needle and syringe method. Then the minimum possible time after the registration profile, pipes alongside ice were sent to the laboratory of Agricultural Jihad in Chahar Mahal and Bakhtiari state. After blood sampling, milk samples were taken within the 50-mL bottles. The overall profile such as the region, sheep and farm numbers were recorded on bottles.

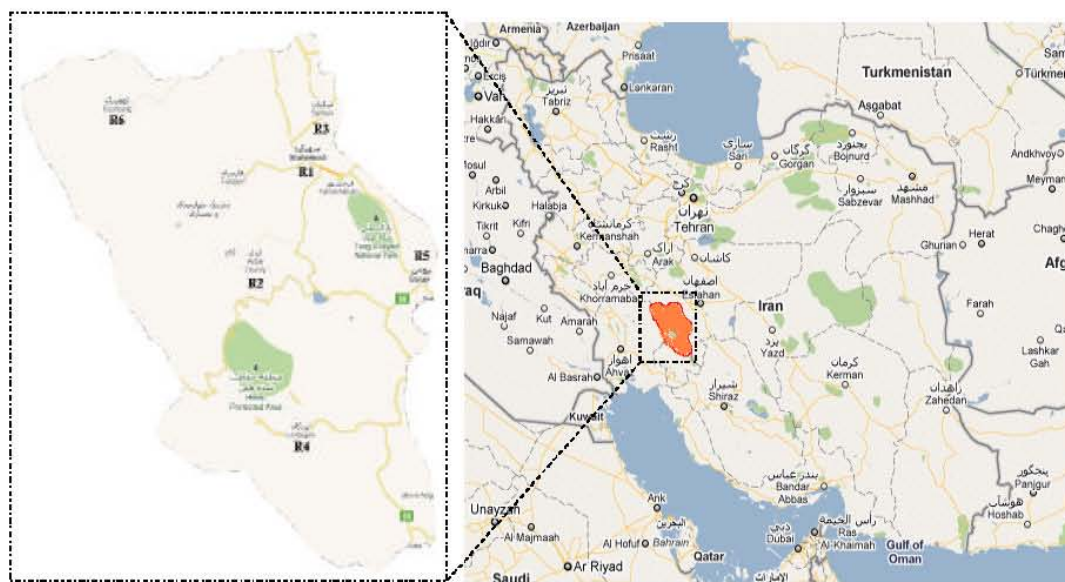


Fig. 1: Geographical map of studied area in Chahar Mahal and Bakhtiari province, west of Iran as map name. The study area is magnified in the left side of map. Key to numbered counties on the map is follows: R1=Shahreکرد, R2=Ardel, R3=Farsan, R4=Lordegan, R5=Brujen and R6=Koohrang

Analyses: Blood samples collected after 8 h to 10 h using a standard procedure and were stored at -2°C . Serum for analysis was obtained by centrifugation at 3000 rpm for 5 minutes. Selenium of diets was measured according to wet digestion method [31]. 5 grams of samples were dried at 100°C for 48 hr. Dried samples were homogenized using an agate pestle and stored in pre cleaned polyethylene bottles until analysis. 6 ml of concentrated nitric oxide was added to bottles content and solution then heated for 6-12 hours at 70°C . After cooling the solution, the amount of 15-10 ml distilled water was added to the container. 1 ml of solution was placed in the reaction balloons and was analyzed after diluted with 9 ml 1.5% chloric acid.

For analysis of serum and milk samples, the amount of a milliliter of milk and serum samples in 16 ml mixture of nitric and perchloric acids (HNO_3 and $\text{ClHO}_4 = 3:1$ v/v, respectively) was digested and procedure was completed by adding distilled water to 25 ml volume.

Selenium in samples was determined by electro-thermal atomic absorption spectrophotometry, using a Shimadzu AA-680 [32] flame atomic absorption spectrophotometer (AAS) equipped to a 196 nm wavelength to measure selenium.

Statistical Analysis: The all data were subjected to the ANOVA procedures by using the GLM procedure of

SAS software [33], which were appropriate for a randomized complete block design. When significant differences ($P < 0.05$) were detected, mean values was compared post-hoc using the Duncan test. The results are expressed as means and their Standard Error (SE).

RESULTS

To evaluate and compare selenium status of serum and milk of Lori Bakhtiari sheep breed among six regions of Chahar Mahal and Bakhtiari Province and also comparison with standard concentrations of selenium standard selenium concentrations in Sheep's milk and serum is given in Table 1-1.

Also present average test results in two phases (first step in May and second in June) in Table 1-2 are shown. There was significant difference between the mean selenium of diet of Lori-Bakhtiari sheep flocks. R6 animal groups had highest mean selenium of diet, serum and milk ($P < 0.05$). Significant different was related to Se contents of R6 and R2 serum samples in comparison with other groups and also differences were detected in R5 samples when compared with R1 results. Examining the mean milk selenium of Lori Bakhtiari sheep flocks in six areas triple province, difference between milk Se of R6 and R2 than others and Se of R1, R3 and R5 in compared to it's amount in R4 was significant.

Table 1: Selenium concentration of pasture predominant serum and milk samples of sheep in studied province

Area	Selenium concentration	
	Blood ($\mu\text{g L}^{-1}$)	Milk ($\mu\text{g L}^{-1}$)
1	0.0233 ^a ±0.0009	0.0045 ^a ±0.0001
2	0.0348 ^a ±0.0013	0.0057 ^a ±0.0001
3	0.0232 ^a ±0.0007	0.0045 ^a ±0.0002
4	0.0208 ^a ±0.0007	0.0042 ^a ±0.0001
5	0.0284 ^a ±0.0012	0.0049 ^a ±0.0001
6	0.0379 ^a ±0.0015	0.0057 ^a ±0.0001
MS	0.0014	0.0005
Total mean	0.0281±0.0007	0.0049±0.0008
P ****		

^{a, b} Values in the same row and variable with no common superscript differ significantly ($P < 0.05$). values are mean of results of both sampling periods of May and June. Values are means of six (for pasture forage samples) and ten (for blood and milk samples) observations per treatment and their standard errors. Regions of study numbered as R1=Shahrekord, R2=Ardel, R3=Farsan, R4=Lordegan, R5=Bruijen and R6=Koohrang. ^bNS= $P > 0.05$; *= $P < 0.05$; **= $P < 0.01$; ***= $P < 0.001$

Table 2: Standard, red-line and deficient concentrations of selenium in pasture predominant forages, serum and milk samples of Lori Bakhtiari sheep of studied province

Area	Selenium concentration	
	Blood ($\mu\text{g L}^{-1}$)	Milk ($\mu\text{g L}^{-1}$)
Shortage	< 40	< 20
Shortage red-lines	40-80	20-40
Sufficient level	> 80	> 40

Table 3: Correlation between Se concentration averages of diet, blood and milk in both step of May and June

Correlation	Pasture predominant forage	Blood	Milk
Pasture predominant forage	1.000	0.225	0.246
Blood	0.225	1.000	0.813
Milk	0.246	0.813	1.000

All values are significant as $P < 0.0001$

DISCUSSION

Regarding to results of presented study, it was detected that amount of Se in milk and serum of R6 sheep flocks was lesser than standard content which can be considered as a Se deficiency or redline.

Davis *et al.* [34] were studied inorganic selenium tolerance by range type ewes during gestation and lactation by adding six different levels of selenium (0.2=control, 4, 8, 12, 16 and 20 mg kg⁻¹) to diets. They reported that Se concentration of colostrums in control group was 257 $\mu\text{g L}^{-1}$ and while colostrums after diet supplementing with 20 mg kg⁻¹ Se, represents a value of 3542 $\mu\text{g L}^{-1}$. The control group milk had 75 $\mu\text{g L}^{-1}$ values and raised up to 2228 $\mu\text{g L}^{-1}$ when 20 mg/kg se added to diet. Correspondingly, serum value of Se was 74 $\mu\text{g L}^{-1}$, but increased up to 775 $\mu\text{g L}^{-1}$, respectively.

From these reported results and other findings of recent studies, it was concluded that amount of Se in product such as milk, meat and egg have a linear link with its levels in diet [35-39]. Whereas some reports have not observed an effect on calve or lamb performance [40-44]. These differences may be the result of the severity of the deficiency in experimental females. Between those mentioned above, serum is the most important one and as a professional transmitter, play a substantial role in transferring Se from animal diet to human diet.

Selenium is an essential element in the diet of animals and has a variety of roles: for example an anti-oxidant that works in conjunction with vitamin E to prevent and repair cell damage in the body; it is involved in immune function and is necessary for growth and fertility. A deficiency can cause white muscle disease (WMD) in lambs and weaners, scouring, ill thrift and lowered wool production in weaners and hogget's and in some cases infertility in ewes. There are other diseases that cause similar clinical signs and there are also other diseases which may be caused by a combination of low levels of both selenium and vitamin E. Selenium deficiency must be confirmed as the cause of disease before treatment because selenium can be toxic if given in excess amounts. Base on results of current study, the areas where selenium deficiency in sheep is likely to occur are shown in the map below.

Selenium deficiency in grazing sheep can occur in areas which shown on the map. selenium shortage can cause various problems, like white muscle disease (WMD) or nutritional muscular dystrophy which is the most common illness who has seen in lambs between two and six weeks of age after forced exercise, such as that associated with marking. On the one hand, curiously, a varying proportion of lambs in a selenium deficient flock do not exhibit the classical symptoms of WMD and in some cases the proportion may be so high that the WMD problem goes unnoticed. Growth rate and general thrift may, however, be adversely affected, with no apparent cause. Selenium deficiency can, therefore, vary in degree from a total imperceptible reduction in rate of growth to a severe deficiency associated with high levels of mortality. "Ill-Thrift" - depressed growth of young without obvious cause - is a major problem in all forms of animal production and it is now evident that selenium contributes importantly to this problem in grazing animals, particularly after weaning.

Selenium administered to deficient stock, particularly during their first year of life, could significantly prevented from WMD or degenerative changes in skeletal muscle

and in the myocardium of young animals [45-47], improved fertility [21, 22] and also increase seminal quality [48] and also live-weight at first joining, hence the apparent stimulatory effect on the incidence of twinning [49]. Selenium has also been shown to significantly improve fleece weight and fleece quality in deficient animals of all ages [22]. These benefits are likely to arise from direct and indirect influences, the indirect effect being mediated through an increase in live-weight, the direct effect through an influence on follicle function. The fleece is probably one of the most sensitive monitors of the adequacy of selenium in the diet, though variation in fleece weight within a flock makes this difficult to validate in experimental work.

In the area which current study has been made, Lori Bakhtiari sheep had different and low fleece quality and weight that recognized by farmers and after some fleece quality evaluations which is not presented because of strain nobility and variation in fleece weight within flocks.

In addition, the biochemical role of selenium is important. It is involved in enzyme systems, including one called glutathione peroxides [50]. This enzyme influences a variety of functions within the organism [51]. It is this wide range of processes that explains the diversity of symptoms of selenium deficiency in livestock which damages cellular and mitochondrial membranes [8, 52].

Overall, the primary cause of selenium deficiency in growing livestock is a deficiency in Se in the pasture. Selenium deficient areas in Iran have not yet been adequately mapped and while light soils and lush legume-dominant pastures are most often associated with selenium responsive conditions in animals, there are many exceptions. In this regard, climate exerts a very significant effect on the incidence of selenium deficiency, mainly through the influence of rainfall on pasture growth. In conditions of rapid growth, the rate of selenium uptake tends to fall and later, as the plant grows older, selenium becomes further depleted, hence problems may arise both during the active phase of pasture growth and later, when it matures. For these reasons selenium deficiency in livestock is often spasmodic, perhaps appearing only in extraordinary good seasons. Small amounts of <0.5 mg/kg in the soil or <0.1 mg kg⁻¹ in plants are considered insufficient [36, 37]. There are clear correlations between the presence of Se in soil, plants and animal tissues [22, 36, 53].

Although selenium is a substantial element for growing livestock, but it is potentially an extremely toxic substance and must be administered with care. Normal

pasture contains about 0.06 parts of selenium per million of dry matter which, assuming an intake in sheep and goats of one kilogram of dry matter per day, represents an intake of 22 milligrams of selenium per year. Its toxicity, together with problems of even distribution on pasture, restrict the incorporation of selenium into fertilizers and there are only three particular ways of administering selenium to animals - oral dosing, injection or administration of heavy pellets with lodge in the rumen and dissolve over a period of months. One of possible way. The simplest and most convenient is drenching, using a solution of either sodium selenate ($\text{Na}_2 \text{SeO}_4 \cdot 10\text{H}_2\text{O}$, M.Wt. 369) or sodium selenite ($\text{Na}_2 \text{SeO}_3$, M.Wt. 172). The normal dose rate for sheep, beyond 12 weeks of age is 5 milligrams of Se.

On the other hand, Stressed grazing animals are most susceptible to selenium; therefore toxicity may be acute or chronic. Signs of selenium toxicity include respiratory distress, restlessness, blindness, staggering, head pressing, anorexia, salivation, abdominal pain, watery diarrhea, convulsions, paralysis and death.

Forage and soil Se determination is important for the diagnosis of Se deficiency and to know the Se status in a particular region. Several factors affect the concentrations of minerals in forages, such as soil type, the presence of antagonistic elements and contaminants, fertilization, forage species, weather and season and plant maturity. These factors may modify and cancel the possibility for the animals to meet their micro-mineral requirements during the year [54]. Volcanic soils have virtually no Se, but have high levels of sulphur which competes with Se for absorption; the plants that grow in this kind of soil and animals that consume them suffer Se deficiency [34].

There are many programs for preventing selenium shortage in Se deficient regions. The time at which selenium should be administered to sheep in selenium deficient areas can be summarized as follows:

- Five milligrams of Se to ewes one month prior to joining.
- Five milligrams of Se to ewes one month before lambing.
- One milligram of Se to lambs (or 5 mg to ewes) at marking (at four weeks).
- Five milligram of Se to lambs at weaning (16 weeks) and further doses 3, 6, 9 and 12 weeks later as necessary up to joining age.

Supplementation of the pregnant females is a key strategy to reduce losses [12, 39]. Supplementation of animals can be accomplished by incorporating the element in the diet (premixes), water, mineral supplements, intra-ruminal bolus, or injectable solutions. On the one hand, Availability of supplemental selenomethionine is greater than that of selenite [55]. Additionally, selenomethionine is rapidly incorporated into proteins [56, 57] and it is suggested adding Se to sheep diet or soil in forms of selenomethionine. Appropriate supplementation in sheep and goats varies from 0.1 to 0.3ppm (DM basis) in the total diet [58]. With subcutaneous solutions of barium selenate, doses of 0.1mg of Se per kg live weight, treated sheep and lambs maintain adequate levels of the element, while with the same salt offered orally at 0.126 mg kg⁻¹ no benefit was observed [59]. In non-lactating cows, oral administration of 1mg Se/day was insufficient to maintain adequate plasma levels [60, 61], whereas with a diet containing 0.3ppm Se during the dry period and the injection of 50mg of Se and 300 IU of vitamin E, 21 days before parturition, treated cows maintained adequate blood and plasma Se levels [62].

If soils are deficient in Se, low levels will occur in plants and animals. Therefore humans will be affected by this deficiency. The Se needs for humans has been estimated at 60-75 µg day⁻¹. The main source of Se for humans is red meat such as beef (28.1 µg 100 g⁻¹) or chicken (23.9 µg 100 g⁻¹). In egg, the Se concentration is 15.4 µg 50 g⁻¹. Milk and its derivatives provide low levels of the element. Grains and cereals provide medium and low quantities of Se, fruit and vegetables were virtually non-contributors [35]. In regions deficient in Se, deficiencies are presented in both humans and animals, with forms of endemic cardiomyopathy being reported in certain regions of world; osteoarthropathy in Northeast Asia [8, 50] and hypothyroidism with associated myxedema, in populations of Central Africa [35, 63, 64]. In the UK, particularly in Scotland, cases of low fertility in men have been diagnosed, caused by the low semen quality after consumption of diets with half of the Se requirements [48].

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