

## EFFECT OF SELENIUM DEFICIENCY ON OVINE IMMUNE STATUS, FREE RADICALS PRODUCTION AND ITS CORRELATION TO DISEASE OCCURRENCE

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### ABSTRACT

*This study have been applied on a total of fifty sheep of both sexes selected from three different sheep flocks, in order to declare the effects of selenium deficiency on the immune junction, production of free radicals and incidence of some disease conditions. The selected animals have been allotted into two groups, Group -1 involved those clinically and apparently healthy animals (n = 10) and were served as control group. While, group -2 involved those animals (n = 40) that had the clinical sign of emaciation, rough coat and poor body condition.*

*Two blood samples (heparinized and non-heparinized) have been obtained from each animal in both groups and were subjected to the laboratory investigations. The obtained biochemical data revealed a significant reduction in the mean values of nitric oxide, T<sub>3</sub>, total lipids and triglycerides associated with significant elevations in the mean values of Malondialdehyde and T<sub>4</sub> in selenium-deficient sheep in comparison with those of healthy ones.*

*Determination of the enzymatic activities particularly catalase, superoxide dismutase and glucose-6-phosphate dehydrogenase have been carried out, and the obtained results revealed that selenium-deficient sheep had a reduced values of these enzymes if compared with those of healthy ones. This reduction might be attributed to the over consumption of these antioxidants resulted from increased levels of the free radicals in the cells of selenium-deficient sheep. Moreover, selenium-deficient sheep had a lower values for blood-selenium, immunoglobulins (IgM and IgG) which may result in increased risk when those deficient animals are exposed to a further disease problem.*

*In addition, cellular examination of milk samples revealed that, selenium deficient-sheep had a higher somatic cell counts than those of the healthy sheep, indicating*

some sort of a sub-clinical form of mastitis in those sheep. Diet analysis revealed that the mean values of selenium were very low than the recommended values for the animals.

In conclusion, selenium deficiency is associated with decreased immunoglobulins, increasing free radicals production with consequent cellular damage and increasing risk of diseases

## INTRODUCTION

Selenium is an essential element for growth, reproduction, and diseases prevention in all animals. Its primary function is to protect cell membranes and proteins from damaging chemicals that are formed during normal metabolism. Body selenium concentrations are directly related to dietary intake of selenium in plant products normally fed to livestock (Gates and Johnson, 2002).

Selenium is a component of the glutathion peroxidase enzyme. This enzyme is important in protecting the integrity of cell membranes. During normal metabolism, the cell produces reactive forms of oxygen (peroxides) which, if not altered, will damage the unsaturated fatty acids found in the cell membrane. Membrane damage will disrupt cell function and adversely affect the animal health (Gates and Johnson, 2002).

This selenium-dependent enzyme catalyzes the reactions that aid in the reduction of both inorganic and organic hydro-peroxides. Vitamin E and selenium through the glutathion peroxidase enzyme are involved in intracellular defenses against oxidant damage (Debski et al., 1992).

At least eleven selenoproteins have been characterized, and there is evidence that additional selenoproteins exist. Most of the biologically active  $T_3$  (triiodothyronine) in the circulation and inside cells is created by the removal of one iodine atom from  $T_4$  (thyroxine). In a reaction catalyzed by selenium-dependent iodothyronine deiodinase enzymes three different selenium-dependent iodothyronine deiodinases (types 1, 2 and 3) can both activate and inactivate thyroid hormone, making selenium an essential element for normal development, growth, and metabolism through the regulation of thyroid hormones (Larsen, 1998; and Holben & Smith, 1999).

Selenium deficiency has been associated with impaired function of the immune system. Moreover, selenium supplementation in individuals who are not overtly selenium deficient appears to stimulate the immune response. It is also indicated by many authors that selenium plays a role in regulating the expression of cell signaling molecules called cytokines which orchestrate the immune response. Selenium deficiency suppresses the immune system response, interfering

with its ability to fight infection and lowering resistance to disease. These problems for the newborn can mean the difference in their survival or death (**Combs and Gray, 1998**).

Selenium affects not only polymorph nuclear neutrophil function, but also lymphocyte proliferation, cytotoxicity, and antibody production (**Grasso et al., 1990**). Impaired bovine lymphocyte function and signaling in response to selenium deficiency may cause the delayed influx of polymorphnuclear (PMN) cells to the mammary gland observed in severe clinical signs in selenium deficient animal challenged with *E. coli* (**Erskine et al., 1989**). Higher IgG concentrations also were observed in the selenium-supplemented ponies **Knight and Tyznik (1990)**.

In addition, Selenium supplementation has been linked to lower herd somatic cell counts and has been shown to decrease the prevalence and severity of clinical mastitis (**Erskine et al., 1989**).

The content of selenium in blood is a reliable indicator of the selenium status in animals; and the values less than 0.04 ppm, 0.05 ppm, and 0.07ppm in beef, sheep and cows, respectively were reported as a deficient values (**Combs and Gray, 1998**). Ovine blood selenium concentration less than 0.04 ppm are considered diagnostic of selenium deficiency (**Whanger et al., 1977**).

Consequently, this study was designed in order to declare the effects of selenium deficiency on the immune function, production of free radicals and occurrence of some disease conditions in sheep.

## **MATERIALS AND METHODS**

### **Animals :**

Three different sheep flocks have been examined and a total of 50 sheep of both sexes, aging 2-3 years were selected for this study. These animals were raised freely on grasses and other field stubbles in a new reclaimed areas and sandy soil. The selected animals have been kept under close observation and were examined clinically according to **Kelly (1984)**. The examined animals have been allotted into two groups, Group -1 involved those clinically and apparently healthy animals (n = 10) and were served as control group; Meanwhile, group -2 involved those animals (n = 40) that had the clinical sign of emaciation, rough coat, low reproductive performance, shuffling movements and poor body condition.

### **Samples and sampling protocol :**

Two types of blood samples have been submitted from each animal and were subjected for laboratory investigations. The first sample was obtained on heparinized tubes and was used immediately for determination of enzymatic activities. The second sample was obtained on non hepari-

nized tubes for obtaining blood serum which was kept frozen until biochemical analysis for the selected parameters. The blood samples were obtained from the diseased as well as the apparently healthy sheep. A randomized milk samples were obtained from a number of sheep and were used for determination of somatic cell count as well as biochemical analysis for some parameters. Also, a representative samples were obtained from the animal s diet and were subjected to analysis.

#### **Biochemical analysis**

Blood sera samples were analyzed For the selected parameters particularly selenium by using atomic absorption spectrophotometer. Immunoglobulines IgG and IgM were measured by using Gel Electrophoresis according to the methods adopted by **Stegmann et al. (1987)**.

Biochemical analysis for determination of the enzymatic activities in whole blood samples for catalase enzyme, superoxide dismutase and GSH were carried out according to the methods described by **Cohen et al., (1970)**, **Misra and Fridovich, (1972)** **Ellmans's, (1959)**, respectively. Meanwhile, blood sera samples were subjected to the biochemical analysis for determination of nitric oxide (**Privat et al., 1997**), triglycerides (**Young and Postaner, 1975**), total cholesterol (**Decg and Zeigenohrm, 1982**), high density lipoprotein (**Warnick et al.,1983**), Low density lipoprotein (**Friedwald et al.,1972**), serum malondialdehyde (**Daper and Hadly, 1990**), T<sub>3</sub> (**Cooper,1982**), T<sub>4</sub> (**Schall et al., 1978**) and total lipids (**Frings and Dunn, 1970**). In addition somatic cell count in milk was carried out using Skar s method (**A.P.H.A., 1985**).

#### **Ration analysis :**

The obtained samples were analyzed by wet ashing technique for determination of selenium concentration (**A.O.A.C., 1984**).

#### **Statistical analysis :**

The mean values and standard error were calculated for the obtained data, and the significances for all means have been carried out by applying t-test using the SPSS computer program. The values have been calculated according to **Snedecor and Cochran (1989)**.

## **RESULTS**

The clinical examination revealed that, the animals of the 2<sup>nd</sup> group showed poor performance with the major clinical signs of cough, rough coat, associated with snuffling. In addition, there were a low reproductive performance, shuffling movements, diarrhea and ill-thrift.

The obtained results of biochemical analysis revealed a significant decrease in the mean val-

ues of nitric oxide (NO), total lipids, triglycerides (TG) and  $T_3$ . Meanwhile, there is an elevation in the mean values of malondialdehyde (MDA), cholesterol, low density lipoproteins (LDL), and thyroxin ( $T_4$ ) in blood serum of sheep with selenium deficiency (table, 1).

Meanwhile, enzymatic activities were summarized in Table (2) which exhibits a significant decrease in the level of catalase (CA), superoxide dismutase (SOD), reduced glutathione (GSH), and glucose-6-phosphate dehydrogenase (G6p-D) levels in erythrocytes of sheep with selenium deficiency.

The obtained results of blood sera analysis for selenium concentrations and immunoglobulins, particularly IgM and IgG and cellular examination of milk samples for the somatic cell counts were expressed in table (3). The results revealed a significant reduction in the mean values of selenium, IgM and IgG in selenium-deficient sheep when compared with apparently healthy animals, associated with significant elevations in the mean values of milk somatic cell counts.

The obtained results of diet analysis revealed that selenium concentration was lower than the recommended requirement for the animal; and the obtained value was 0.056 ppm.

## DISCUSSION

Since the selenium intake is considered to depend on the selenium content in feed stuffs supplied to the animals; therefore it was crucial to analyze the diet for selenium content. The obtained results of diet analysis revealed that selenium concentration was lower than the recommended requirement for the animals (0.056 Vs 0.1 ppm). As a dietary level of 0.1 ppm selenium is quite sufficient to prevent the signs of deficiency in most animal species (**Harrison and Conrad, 1984**).

It was postulated by (**Fang and Yang 2002**) that, free radicals (superoxide, nitric oxide, and hydroxyl radicals) and other reactive species (hydrogen peroxide, peroxynitrite, and hypochlorous acid) are produced in the body, primarily as a result of aerobic metabolism. Antioxidants (glutathione, arginine, citrulline, taurine, creatine, selenium, zinc, and vitamin E) and antioxidant enzymes (superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidases) exert synergistic actions in scavenging free radicals and consequently protecting the body cells and tissues from the deleterious effect of free radicals.

Nitric oxide (NO) is synthesized by the enzyme nitric oxide synthetase, which converts the amino acid L-arginine to citrulline and nitric oxide. Nitric oxide plays a role in cells communicating with one another (signal transduction), and it has a critical role in the host response to in-

fection. In this regard, it appears that the toxic properties of nitric oxide have been harnessed by the immune system to kill or at least to slow the growth of invading organisms (**Marletta and Spiering, 2003**).

The obtained results revealed a significant reduction in the mean values of nitric oxide and glucose-6-phosphate dehydrogenase in selenium-deficient sheep, the results which are in agreement with those obtained by **Liu et al. (2000)** and **Leopold et al. (2003)** who found that, selenium solution could increase the content of NO in the mice's blood sera and may be associated with the increase in antioxidative activity, suppression of free radical's intervention, and the excessive release of NO. Oxidative stress may be induced by generation of reactive oxygen species (ROS) and other free radicals. The generation of these reactive oxygen species is known to be associated with a decrease in antioxidant levels (**Yildirim et al., 2003**).

**Leopold et al. (2003)** reported that glucose-6-phosphate dehydrogenase, the first enzyme of the pentose phosphate pathway, is the principal intracellular source of NADPH. NADPH is utilized as a cofactor by vascular endothelial cell nitric oxide synthetase to generate nitric oxide.

Meanwhile, it was revealed by **Grasso et al. (1990)** that, the production of hydrogen peroxide increased two folds in the neutrophils obtained from cows fed the selenium deficient diet compared with selenium supplemented diet. Although hydrogen peroxide is necessary for most of the oxidative killing mechanisms used by neutrophils excess hydrogen peroxide potentially can be detrimental to the cell itself. Increased concentration of free radicals may be formed, which can interact with other cellular components, resulting in the formation of lipid peroxides.

Most of the mammalian selenium is incorporated or bound by certain proteins, selenoproteins or selenium-binding proteins, respectively (**Evenson and Sunde, 1988** and **Burk et al., 1991**). One of the most important selenium protein in mammals is glutathion peroxidase (**Zachara, 1992**). Glutathion peroxidase catalyzes the reduction of hydrogen peroxides and organic peroxides and thus protecting the membranes against oxidative damage (**Ullrey, 1987**).

Selenium and vitamin E act synergistically and having an effect on the immune system. They modify the interactions of macrophages and lymphocytes (**Afzal et al., 1984**) or act as antioxidants on the cells involved in the immunological reactions (**Reffet et al., 1988**). Lymphocytes are regarded as the cell population most sensitive to peroxidation due to the high free lipid acid content of their membranes; (**Nemec et al., 1990**).

Blood leucocytes have significantly higher GSH-PX activity cell than do erythrocytes (**Scholz and Hutchinson, 1979**). Glutathion peroxidase may be involved in phagocytosis by leucocytes which is a major immune mechanism of bacterial clearance (**Erskine et al., 1987**). Decreased GSH-PX activity in phagocytic cells has been reported in selenium - deficient animals (**Serfass &**

Ganther ,1976) and has been associated with reduced bactericidal capacity of neutrophils (Serfass & Ganther , 1976; Gyang et al., 1984; Kanafani and Martin, 1985).

The obtained results revealed a significant increase in  $T_4$  and significant decrease in  $T_3$  in selenium-deficient sheep. Such results could be attributed to the reduction in deiodinase type I (DI-I) activities in the peripheral tissues in selenium-deficient animals (Beech et al. 1995). The reduction in deiodinase activity in the peripheral tissues results in decreased rate of deiodinisation of  $T_4$  to  $T_3$  with consequent elevation of circulating  $T_4$  (Arthur et al. 1990).

In this study selenium-deficient sheep showed significant reduction in the mean values of total lipids and triglycerides associated with significant elevations in the mean values of cholesterol, low density lipoprotein and malondialdehyde. Meanwhile High density lipoproteins showed non significant variations between the healthy and selenium deficient sheep.

The reduction in total lipids in selenium-deficient sheep could be attributed to the reduction in lipid absorption from small intestine due to pancreatic atrophy that may have resulted from selenium deficiency with consequent reduction in pancreatic enzymes necessary for lipid absorption (Bunk and Combs, 1981).

Moreover, it was reported by Mazur et al. (1996) that selenium deficiency have resulted in increased concentrations of plasma cholesterol and apolipoprotein E. Meanwhile, selenium deficiency in combination with vitamin E deficiency leads to an increase in plasma low density lipoprotein (LDL) and apolipoprotein B concentrations.

The obtained results of this study revealed that there is a direct correlation between the blood values of selenium and immunoglobulins. Reduced selenium concentrations in the blood was associated with reduced IgM and IgG in selenium-deficient sheep when compared with their levels in healthy ones. The results which are in concern with those reported by Finch and Turner (1986), Knight and Tyznik (1990), Larsen et al. (1988), Mavromatis et al.(1990), and Spallholz et al.(1973).

There is an evidence indicating an enhanced immunoglobulin M and immunoglobulin G antibody titers in mice fed selenium Spallholz et al. (1973). In addition, the results obtained by Finch and Turner (1986) showed that marginally selenium deficient lambs are able to elicit strong antibody titers to a bacterial antigen and that supplemented with selenium produces, at best, a marginal enhancement of the responses observed.

Clinically, it was observed in this study that, five cases of mastitis have been observed in selenium-deficient sheep and associated with highly significant increase in the mean values of somatic cell count in comparison with the values in healthy ones. Selenium deficiency has been associated with increased prevalence of mastitis in dairy cows and other lactating mammals

**(Grasso et al., 1990)**. Goats and cattle consuming selenium deficient diets have milk with high somatic cell counts and decreased milk production **(Erskine et al., 1989)**. This indicates that selenium plays an important role in mammary resistance to infection, but the precise mechanism is still unknown.

Such results could be explained as, the generated peroxides leads to damage of the epithelial cells in which the neutrophils have accumulated **(Badwey and Karnovsky, 1980; Fanton and Ward, 1982)**. In the mammary gland, these damage to the epithelial tissues may lead to an irreversible loss of secretory tissue and reduction of milk yield from the gland **(Grasso et al., 1990)**.

It could be concluded from this study that, Understanding free radicals biology is necessary for designing an optimal nutritional countermeasure against cytotoxicity. The knowledge of enzymatic and non-enzymatic oxidative defense mechanisms will serve as a guiding principle for establishing the most effective nutrition support to ensure the biological safety. Appropriate dietary interventions may reduce the potentially damaging effects of free radicals generated during metabolism and various physiological conditions. The results indicates that selenium enhances the antioxidant capacity in animals.



Table (1): The mean values of some blood biochemical parameters of selenium-deficient and clinically healthy sheep.

Biochemical parameters	Control animals (Group -1)	Selenium-deficient animals (Group -2)
Nitric oxide (nmol /ml)	48.59 ± 0.82	13.05 ± 0.63*
MDA (µmol/L)	0.44 ± 0.07	1.41 ± 0.1*
T4 (ng/ml)	35.33 ± 0.64	55.26 ± 0.61*
T3 (ng/ml)	17.79 ± 0.92	2.38 ± 0.58*
Total lipids (mg/dL)	427.2 ± 21	322.6 ± 13 *
Cholesterol (mg/dL)	95.48 ± 5.9	117.64 ± 1.7*
Triglycerides (mg/dL)	249.98 ± 0.7	233.43 ± 1.4*
HDL (mg/dL)	45.1 ± 2.4	44.7 ± 1.1
I.DL (mg/dL)	134.94 ± .46	166.28 ± 1.06*

\*Means are significantly different at the level ( $P \leq 0.05$ )

Table (2): The mean values of some enzymatic activities in the blood of selenium-deficient and clinically healthy sheep.

Biochemical parameters	Control animals (Group -1)	Selenium-deficient animals (Group -2)
Catalase (nmol /ml)	48.59 ± 0.82	13.05 ± 0.63*
SOD (µmol/L)	1.41 ± 0.1	0.44 ± 0.07*
GSH (ng/mL)	55.33 ± 0.64	35.26 ± 0.61*
G6P-D (U/10 <sup>12</sup> RBCs)	257.3 ± 5.2	210.8 ± 8.9*

\*Means are significantly different at the level ( $P \leq 0.05$ )

Table (3): The mean values of selenium and immunoglobulines in the blood, and somatic cell count in the milk of selenium-deficient and clinically healthy sheep.

Biochemical parameters	Control animals (Group -1)	Selenium-deficient animals (Group -2)
Selenium ppm	69.56 ± 3.6	15.25 ± 2.1*
IgG mg/dl	2156.4 ± 30.6	1325.5 ± 23.2*
IgM mg/dl	196.2 ± 12.5	132.2 ± 6.8*
Somatic cell count	73.6 × 10 <sup>3</sup> ± 6.4	267.6 × 10 <sup>3</sup> ± 12.36.4*

\*Means are significantly different at the level ( $P \leq 0.05$ )

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## الملخص العربى

## تأثير نقص السيلينيوم على الحالة المناعية فى الأغنام وتكوين الشقائق الحرة النشطة وعلاقتها بحدوث الأمراض

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أجريت هذه الدراسة على ثلاث قطعان من الأغنام وقد تم إختيار خمسين حيوان من كلا الخنسين تراوحت أعمارهم فيما بين عامين إلى ثلاثة أعوام. وقد أجريت هذه الدراسة بفرض إستبيان تأثير نقص عنصر السيلينيوم فى دم الأغنام على الحالة المناعية وكذلك تكوين المواد النشطة المؤكسدة وحدثت بعض الأمراض، وقد قسمت هذه الحيوانات إلى مجموعتين، حيث ضمت المجموعة الأولى عشر حيوانات سليمة إكلينيكياً واستخدمت كمجموعة ضابطة، أما المجموعة الثانية فقد ضمت أربعين حيوان تعاني من بعض العلامات المرضية الظاهرية مثل الهزال وجفاف الجلد وبعض المشاكل المتعلقة بالجهاز الحركى للحيوانات.

تم أخذ عينات دم من حيوانات المجموعتين وذلك لإجراء التحاليل المعملية اللازمة، كما تم أخذ عينات عشوائية من العلائق المستخدمة فى تغذية حيوانات المجموعتين وذلك لإجراء التحاليل المعملية اللازمة لتحديد مستوى عنصر السيلينيوم.

كما أظهرت النتائج المعملية حدوث نقص معنوى فى مستوى السيلينيوم فى الأعلاف التى تتغذى عليه هذه الحيوانات عن المعدلات القياسية.

هذا وقد أظهرت النتائج المعملية حدوث نقص معنوى فى مستويات كل من أكسيد النترك، الشيروكسين، الليبيدات الكلية والجليسريدات الثلاثية مع حدوث زيادة معنوية فى مستويات MDA و T4 فى دم الحيوانات التى تعاني من نقص عنصر السيلينيوم إذا ما قورنت بمستويات تلك العناصر فى دم الحيوانات السليمة، كما أظهرت نتائج التحاليل البيوكيميائية للدم حدوث نقص معنوى فى نشاط بعض الإنزيمات مثل الكاتاليز والجلوتاثيون المختزل و SOD التى تعمل كمضاد للأكسدة فى أنسجة الجسم وذلك بسبب تراكم المواد النشطة المؤكسدة والتى ترجع إلى النقص فى مستوى السيلينيوم.

هذا وقد أظهرت النتائج المعملية حدوث نقص معنوى فى مستويات الأجسام المناعية IgG, IgM، والتى صاحبت نقص السيلينيوم فى الدم مما ساعد على تعرض بعض الحيوانات للإصابة ببعض المشكلات المرضية مثل إلتهاب الصرع، بالإضافة إلى ذلك فقد تم إجراء الفحص الخلوى لعينات اللبن والتى أفادت حدوث زيادة معنوية فى عدد Somatic cell فى الأغنام التى تعاني من نقص السيلينيوم.

وبناء على ماتقدم، يمكننا أن نستخلص أن نقص السيلينيوم يؤدي إلى آثار ضارة بالحالة المناعية للحيوان وتكوين المواد المؤكسدة النشطة داخل الأنسجة مما يؤدي إلى خفض مناعة الحيوان ويقلل مقاومته للعديد من الأمراض.