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Induction of vitamin E and selenium deficiency in Awassi ewes and their newborn

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CERTIFICATION

We certify that the thesis entitled **((Induction of vitamin E and selenium deficiency in Awassi ewes and their newborn))** has been prepared under our supervision at the College of Veterinary Medicine / University of Baghdad in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Internal and Preventive Veterinary Medicine.

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Dedication

To my family

Haidar

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Summary

Twenty eight Awassi ewes and 24 newborn lambs were used to carry out clinical, hematological and immunological studies of experimentally induced vitamin E and selenium deficiency. The work lasted for 9 months started from 1.3.2011 to 1.12.2011 and involved the following steps:

The first step: Selection and grouping of animals:

Animals of the study belong to State Board of Agricultural Research / Ministry of Agriculture, raising system of animals is of intensive type. Forty Awassi ewes were selected, 20 ewes were taken from a herd of 120 ewes which were virgin (18 months old) and not vaccinated with Rev 1 vaccine , other 20 ewes were selected from a herd of an open animals (26 months old) and vaccinated with Rev 1 vaccine.

The second step: Carrying out an estrus synchronization:

Before estrus synchronization started, the animals' uterine health was examined by using a real time B-mode scanner equipped with a 7.5MHz linear array prostate transducer which shows a rectangular image. Estrus synchronization was performed by insertion of a vaginal sponges.

According to the deficient diet, treatments and vaccine used the groups of ewes were allocated as follows: Animals in group (1) which were 26 months old were fed the deficient diet and vaccinated with Rev 1 vaccine, Animals in group (2) which were 18 months old were fed the deficient diet only, Animals in group (3) which were 26 months old were vaccinated with Rev 1 vaccine only and Animals in group (4) which were 18 months old were fed the ordinary diet and left as a control group.

The third step: Induction of vitamin E and selenium deficiency:

Vitamin E and selenium deficiency was induced by feeding a diet consisted of cod liver oil at a concentration of 3% mixed with ground corn 0.5 kg/animal/day, discolored bad quality hay offered all the time and water was offered all the time.

The fourth step: Samples and laboratory work:

Simultaneously with appearance of clinical signs of deficiency in lambs and ewes blood without anticoagulant was collected via jugular venipuncture and serum was separated to estimate selenium and vitamin E levels and creatine kinase and aspartate aminotransferase activities. Whole blood samples with anticoagulant were collected and each sample evaluated the total and differential white blood cell counts, erythrocyte osmotic fragility and phagocytosis.

Colostrum was collected within the first day of parturition to estimate selenium and vitamin E levels. Milk samples were collected after the colostrum was completely milked from the udder for estimation of selenium and vitamin E levels and performing the somatic cell count.

The results showed that the clinical signs of vitamin E and selenium deficiency in this study were mainly sudden death in 4 lambs out of 14 lambs born to deficient dams. Other 10 lambs born to deficient ewes which remained live showed a variable signs included inability to suckle, arched back, weakness, dullness, emaciation and recumbency. The postmortem changes in the dead lambs were also recorded and were mainly paleness and atrophy of thigh muscle and paleness of heart muscle.

The clinical signs in deficient ewes were mainly loss of body weight, decreased milk production and loss of wool. Weakness, dullness and recumbency were also found.

Estimation of vitamin E and selenium levels in serum, colostrum and milk revealed lower levels in deficient animals than in healthy animals with significant differences ($P<0.05$) between different groups of ewes and lambs.

Phagocytic index showed lower values of deficient animals than in healthy animals with significant differences ($P<0.05$) between different groups of lambs and ewes.

Erythrocyte osmotic fragility test revealed that start hemolysis and complete hemolysis were high in deficient animals as compared to healthy animals with significant differences ($P<0.05$) between different groups.

The results showed that there was a reduction in total white blood cell count in deficient animals as compared to healthy animals while in differential white blood cell count, the results showed a reduction in lymphocyte count and increase in neutrophil, eosinophil and basophil percentages in deficient animals in comparison to healthy animals with significant differences ($P<0.05$) between different groups.

The results revealed an increase in serum activities of creatine kinase and aspartate aminotransferase in deficient animals as compared to healthy animals with significant differences ($P<0.05$) between different groups. Milk samples of deficient ewes showed a higher somatic cell count as compared to healthy ewes with significant differences ($P<0.05$) between different groups.

List of Contents

List of Tables

List of figures

Chapter One

Introduction

Vitamin E is the generic descriptor for two families of compounds, the tocopherols and the tocotrienols. The different vitamers (compounds having similar vitamin activity) have different biologic potencies; the most active is D-α-tocopherol, and it is usual to express vitamin E intake in milligrams of D-α-tocopherol equivalents. Synthetic DL-α-tocopherol does not have the same biologic potency as the naturally occurring compound (Murray *et al*., 2003) a.

Selenium was discovered in 1817 by the Swedish chemist Berzelius. Berzelius considered that the new element was kindred to tellurium, which was named in honor of the Earth (in Latin the name of our planet is Tellurus). On analogy of this he named the new element Selenium (Selene is the goddess of the moon in Greek mythology). Selenium is an essential trace element for humans and animals whose dietary intake is not sufficient in many parts of the world (Molnar *et al*., 1998).

Several diseases of sheep such as nutritional (enzootic) muscular dystrophy, ill-thrift (selenium responsive), reproductive inefficiency (selenium responsive) and bone marrow abnormalities are associated with a deficiency of either selenium or vitamin E alone or in combination, usually in association with predisposing factors such as dietary polyunsaturated fatty acids, unaccustomed exercise and rapid growth in young animals (Radostits *et al*., 2007).

Selenium and vitamin E deficiencies occur when animals are fed poor-quality hay or straw and lack access to pasture. Diets high in polyunsaturated fatty acids contribute to the development of neuromuscular disease by increasing the requirement for vitamin E (Pugh, 2002).

In many circumstances, selenium deficiency cannot be considered alone and must be addressed in concert with vitamin E deficiency. The characteristic clinical outcome of both is white muscle disease, and this reflects their complementary, although independent, roles as cellular antioxidants (Hidiroglou *et al*., 1992). White muscle disease or nutritional muscular dystrophy in lambs is an enzootic and nutritional disease, which results from selenium and vitamin E deficiencies. This disease has been reported in many countries of the world (Osame *et al*., 1990).

Biological functions of selenium are complemented by vitamin E, which also shows the effects of a cellular antioxidant. Therefore, some clinical manifestations of hypovitaminosis E and selenium deficiency are very similar. Therapy and prophylaxis of these disorders usually consist of the administration of both substances together (Allison and Laven, 2000).

In order to well understand the effects of vitamin E and selenium deficiency in Awassi sheep the following goals have been planned:

1.Study the effects of vitamin E and selenium deficiency on hematological and immunological parameters in ewes and their newborn lambs.

2.Reporting the clinical signs and postmortem changes caused by the deficiency in ewes and their newborn lambs.

Chapter Two

Review of Literatures

2-1-Sources of vitamin E:

Vitamin E occurs naturally in varying proportions as a mixture of alpha- (the most potent), beta-, gamma- and delta-, and possibly other tocopherols. Green plants and cereal grains are good sources of vitamin E and a primary dietary deficiency is unlikely when animals are grazed on green pasture, fed on a high grain ration or good quality hay (Blood and Henderson , 1968).

Feedstuffs, such as green grass and fresh hay ,contain a high concentration of tocopherol, but tocopherol content is decreased by oxidative damage during long storage (Putnam and Comber, 1987).

The primary source of vitamin E for dairy cows is forage. Processing and storage of forage can result in considerable loss of vitamin E. Serum vitamin E levels in cows have been demonstrated to decrease seasonally, corresponding approximately with length of feed storage (Braun *et al*., 1991).

The amount of vitamin E in feedstuffs is dependent upon several factors such as: plant species, maturity at harvesting and the conditions of storage and processing (Ullrey, 1981).Vitamin E as an ingredient is generally poor under conditions that promote oxidation of feedstuffs such as: heat, moisture, oxygen, oxidizing fats, and trace minerals and prolonged exposure to sunlight increases the loss of vitamin E .Silage and hay contain about 80% less alpha-tocopherol compared to fresh forage (McDowell, 1989).

Tocopherol content of forages varied widely and was affected by: (a) stage of maturity at time of cutting, (b) the period of time from cutting to dehydration (losses during drying in the swath reached about 60% within four days) and (c) storage in which losses of 50% occurred in a month (Bunnell, *et al.* 1968).

Oxidation of vitamin E in concentrates increases following grinding, mixing with minerals, the addition of fat and pelleting. Corn is variable in alpha-tocopherol (Jordan, 1970).

The incidence and/or severity of vitamin E-responsive deficiency diseases seems to be influenced by placental and mammary gland transfer of alpha-tocopherol (Hidiroglou *et al*., 1972). Placental transfer of alpha-tocopherol is very limited in cattle (Van Saun *et al*., 1989), sheep (Hidiroglou *et al*., 1970 and Pehrson *et al*., 1990), humans (Ostrea *et al*., 1986), and rats (Mason and Bryan, 1940).

2-2-Sources of selenium:

Selenium is microelement ranking among substances with biological activity in intermediate metabolism. It gets into the organism mainly as component of animal diet and it's transplacental transfer is an important factor in the young. The level of absorption and retention of microelement is modulated by its actual level in the organism and it's concentration in the diet and is generally higher for intake of their organic forms (Kuricova *et al*., 2003).Ultimately, the soil is the source of selenium, it enters the food chain through incorporation into vegetable protein as the amino acids selenocysteine and selenomethionine (McKenzie *et al*., 1998 and Whanger, 2002).

Selenium is part of the antioxidant defense system in animals and humans. The available selenium concentration in soil is low in many regions of the world (Steen *et al*., 2008).Natural intake of selenium by ruminants depends primarily

on the geographical position, or more specifically, on selenium concentration in soil. The general and well documented principle of the control of trace element intake by the link soil-plant-animal applies also to selenium (Kamada *et al*., 2000).

Availability of soil selenium to plants depends on soil acidity, structure and degree of aeration. Availability of oxidized forms is higher than that of the reduced form which remains un dissolved in the soil. Selenium is incorporated into plant tissues mostly in the form of selenomethionine present in the protein component of grain. Hence, the content of selenium in grain closely correlates with the content of protein (Pavlata *et al*., 2001) a.

Further factors influencing the content of selenium in plants include the vegetation phase, diluting effects of long-lasting rains, season, and fertilisation with sulphur (Ammerman and Miller, 1975).

Like with most minerals, satisfactory intake of selenium by ruminants depends on the presence of this element in soil and on the potential of plants to utilize it. The occurrence of selenium in soil varies considerably worldwide (Pavlata *et al*., 2002).Forages in many areas of the world do not provide adequate dietary selenium for livestock, whereas in other areas selenium concentrations in some grasses are high and can result in animal toxicity (Herdt *et al*., 2000).

The trace element contents of certain foods and total diets vary widely due to several factors. Climatic condition during the plant growing season, soil pH, redox potential, presence of some inorganic and organic compounds influence the mineral content of foods of plant origin. Other, even more complex factors affect the mineral composition of foods of animal origin. Differences due to the existing various food industrial and kitchen technical characteristics of the

populations of different countries must not be neglected either. For this reason, dietary selenium intake varies substantially across populations (Giovannucci, 1998).

In the majority of circumstances there is a very strong correlation between the concentration of selenium in the geological parent materials and the soils derived from them. The selenium content of most soils is very low at 0.01-2 ppm (world mean is o.4 ppm), but high concentrations of up to 1200 ppm have been reported in some seleniferous areas (Neal, 1995).

Although there is little evidence that selenium is essential for vegetation growth , it is incorporated into the plant structure. Selenium concentrations in plants generally reflect the levels of selenium in the environment such that the same plant species grown over high and low selenium-available soils will contain concentrations reflecting the soil composition. However ,an important factor that may determine whether or not selenium-related health problems in animals and humans is the very wide-ranging ability of different plant species to accumulate selenium (Jacobs, 1989).

Plant species differ in the amounts and concentrations of selenium 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 ppm (part per million) selenium , respectively (Coburn Williams and Mayland, 1992).

Selenium is introduced into the food chain by plants, which absorb inorganic selenium salts from the soil and convert them into organic forms of the element (mainly as selenomethionine), which are then incorporated into proteins. The concentration of selenium in plants varies widely and depends on the

selenium content and characteristics of soil Pavlata *et al*., (2002) and Pavlata *et al*., (2005).

Selenium concentration occurring naturally in forages and grain vary considerably, depending on the plant species and especially the selenium status of the soil from which they were grown (McDowell, 1992).

Selenium is not required by plants; however, it is an essential trace mineral required for adequate nutrition and health of animals, birds, fish and humans. Plants, while not requiring selenium for growth, absorb the oxidized form of selenium from the soil and cycle it to ingesting animals. In general diets containing 0.1 to 0.3 ppm selenium will supply an adequate amount of selenium for animals, birds, fish and humans (Mayland, 1994).

Selenium is not known to be essential for plant growth, but its concentration in forage and hay crops is important to animal health. Plants may contain from none to several thousands ppm of selenium depending upon the plant species, the soil, and the soil parent material (Carter, *et al*. 1968).

In newborn animals and during milk suckling, the availability of selenium depends on that availability in the mother, as selenium permeates placental and mammary barriers. Placental transfer is more effective than the transmission of selenium to the calf through milk (Enjalbert *et al*., 1999).

2-3-Biological roles of selenium and vitamin E:

Vitamin E is essential for such body functions as growth, reproduction, prevention of various diseases, and protection of the integrity of tissues. The metabolic function of selenium is closely linked to vitamin E. Both vitamin E and selenium act to protect biological membranes from oxidative degeneration. The most serious effect of selenium and vitamin E deficiency is tissue degeneration (McDowell *et al*., 1996).

Selenium was first identified in the 1930's as a toxic element to some plants and animals. However, selenium is now known to be required by laboratory animals, food animals, and humans for proper growth and immune function (Schwartz and Foltz, 1957).

Selenium is also an integral component of the Glutathione peroxidase (GSH-Px) in the organism (Pamukçu *et al*., 2001). Glutathione peroxidase neutralises the effects of hydrogen peroxide and lipid hydroperoxide, which cause cell protein destruction and necrosis (Beytut *et al*., 2002).

Selenium is present in all tissues of the organism. Physiological functions of selenium are mediated in particular by selenoproteins. Glutathione peroxidases (GSH-Px 1-4) belong among the most important selenoproteins, whose main function coincides with their antioxidative effect (Underwood and Suttle, 1999).

Biological functions of selenium are complemented by vitamin E, which also shows the effects of a cellular antioxidant. Therefore some clinical manifestations of hypovitaminosis E and selenium deficiency are very similar. Therapy and prophylaxis of these disorders usually consist of the administration of both substances together (Allison and Laven, 2000).

A primary role of vitamin E is as a fat-soluble chain-breaking antioxidant essential for the prevention of lipid peroxidation in cell membranes, particularly during periods of rapid cell growth in the fetal and neonatal animal (Wang and Quinn, 1999). Biological functions of selenium are carried out by a series of selenoproteins whose main role is participation in the removal of reactive forms of oxygen from the organism (Birringer *et al*., 2002).

Subsequently, it was found that vitamin E and selenium are essential for proper health, immunity and reproductive functions of animals, and their inter-relationship became an active and rewarding field of research, as vitamin E and selenium are the micronutrients that share a common biological function. Selenium is a component of glutathione peroxidase enzyme, which destroys free radicals in the cytoplasm, whereas, vitamin E is a non-enzyme scavenger of free radicals that functions as a specific lipid soluble antioxidant in cell membranes. Subsequently, it was confirmed that selenium and vitamin E act synergistically and protect the tissues against oxidative damage. Both selenium and vitamin E have been shown to improve immune responses (Shinde *et al*., 2007).

Selenium is required for the development and expression of non-specific, humoral and cell mediated immune responses. However, the mechanism by which affects the immune system is speculative. Probably, the effects of selenium through glutathione peroxidase and on the cellular levels of reduced glutathione, as well as the ability of selenium to interact with cell membranes, represent the immune-enhancing role of selenium (Shinde *et al*., 2009).

Protection of embryonic cells from free radical damage by supplementing culture media with vitamin E for 7 days immediately after fertilization increased the number of bovine zygotes that developed to expanded blastocysts in vitro (Olson and Seidel, 2000).

Furthermore, embryos cultured in the presence of vitamin E for 5 days between fertilization and transfer developed into larger blastocysts than did embryos cultured in control media, when assessed on day twelve (Arechiga *et al*., 1994).

The main function of vitamin E is as a chain-breaking, free radical trapping antioxidant in cell membranes and plasma lipoproteins. It reacts with the lipid peroxide radicals formed by peroxidation of polyunsaturated fatty acids before they can establish a chain reaction. The tocopheroxyl free radical product is relatively unreactive and ultimately forms nonradical compounds (Murray *et al*., 2003) b.

The importance of the antioxidant roles of vitamin E and selenium is accentuated in ewes grazing spring grass rich in polyunsaturated fatty acids or when the energy density of late-pregnancy concentrate supplements is boosted by polyunsaturated fatty acids-enriched supplements. Nutritional imbalances, such as vitamin E and selenium deficiencies ,which lead to sibling embryo mortality during implantation ,decrease the subsequent fetal growth and birth weight of survivors by restricting placental size (Robinson *et al*., 2002).

Selenium is incorporated into a number of functionally active selenoproteins, including the enzyme glutathione peroxidase which protects cells against damages caused by free radicals and hydroperoxides or lipoperoxides. There are four known forms of Glutathione Peroxidase containing selenium. Selenium glutathione peroxidases contain a covalently bound selenium atom in the form of a selenocysteine molecule in their active center. The substitution of selenocysteine with normal cysteine at the active site of Glutathione Peroxidase has been shown to dramatically reduce its enzymatic activity. There are at least two other similar proteins that do not contain selenium. The forms Glutathione Peroxidase-1 and Glutathione Peroxidase-2 are found in the cytosol , while Glutathione Peroxidase-3 is found in the plasma, and Glutathione Peroxidase-4 performs special functions in the metabolism of phospholipid hydroperoxides (Micke *et al*., 2003).

Vitamin E reacts with peroxide radicals produced from polyunsaturated fatty acids in membrane phospholipids or lipoproteins to yield a stable lipid hydroperoxide. This antioxidant activity of vitamin E in preventing lipid peroxidation may be one of the mechanisms by which vitamin E enhances immunity (Chew, 1996).

Selenium is concentrated in tissues involved in the immune response, such as lymph nodes, spleen and liver, and various components of the immune system have been shown to be impaired if dietary intake of selenium is inadequate (Forencik and Ebringer, 2003).

Vitamin E is an essential antioxidative component of cell membranes that prevents peroxidative damage to the cell membrane and membranes of subcellular organelles by free radicals. Selenium has a biological function related to vitamin E in that selenium is an essential component of glutathione peroxidase (GSHPx), an enzyme involved in detoxification of hydrogen peroxide and lipid hydroperoxides (Rotruck *et al*., 1973).

The antioxidative role becomes very important during the immune response when neutrophils produce large quantities of superoxide and hydrogen peroxide from molecular oxygen to destroy ingested foreign organisms (Ross, 1977).

It is well documented that selenium and vitamin E play important roles in maintaining herd health of dairy cattle (Smith *et al*., 1984). The main pathway for selenium loss from the organism include urine, excrements, milk, and, potentially also, exhaled air (Arova *et al*., 2003). The importance, variety and great number of biological functions played by selenium as a microelement imply the risk that any deficit present as early as during the pregnancy of the mother may have a negative impact on the development of the foetus and the

health of the calf. It has been proved that selenium cross the placental and mammary barrier. Sufficient selenium saturation of pregnant animals is very important also with respect to the needs of the young during intrauterine and early postnatal development (Pavlata *et al*., 2003).

Pavlata *et al*., (2004) stated that selenium affects milk and colostrum quality and incidence of mastitis was decreased when dairy cows were injected with selenium and vitamin E.

Selenium is an essential element required in small amounts by animals and humans for the basic functions of life. It has several structural and enzymatic roles, of which, the best known is as an antioxidant through the enzyme glutathione. Glutathione peroxidase is believed to be a critical enzyme in the human body that combats oxidative damage at the cellular level. The enzyme in conjunction with vitamin E catalyses the reduction of hydrogen peroxide and a range of lipid hydroperoxides to protect biological membranes from oxidative degradation (Gropper, *et al.* 2005 and Pechova, *et al.* 2008).

A dietary deficiency of vitamin E reduces the activities of hepatic catalase, glutathione peroxidases, and glutathione reductase (Chow, *et al*., 1969). (Carr, *et al*.,2000) reported that vitamin E deficiency induces liver lipid peroxidation, and causes neurologic and cardiovascular disorders, all of which can be reversed by dietary vitamin E supplementation.

In support of the critical antioxidant role of vitamin E, (Yokota *et al*., 2001) recently demonstrated increases in brain lipid peroxidation and neurodegeneration in mice with a deficiency of alpha-tocopherol transfer protein.

Along with selenium, vitamin E ranks among very important antioxidant agents protecting the organism from the effect of reactive oxygen forms. As an extinguisher of peroxidation reactions in membranes, vitamin E is probably the

most important antioxidant in cell membranes (Scott,1980 and Rice and Kennedy, 1988).

Vitamin E and selenium are micronutrients that share a common biological function in the animal body. The antioxidant effect of selenium depends mainly on glutathione peroxidase (GSH-Px), in which selenium is contained. Vitamin E and glutathione peroxidase operate at different sites in the cell. The function-site for glutathione peroxidase is cell cytosol and vitamin E operates within lipid membranes. One important function of both systems is protection of polyunsaturated fatty acids (PUFA) in membranes, which are very sensitive to the effect of reactive oxygen forms. Hypovitaminosis E in animals is associated with polyunsaturated fatty acids (PUFA) metabolism alteration, which may subsequently lead to impaired function of cells such as polymorphonuclear neutrophils, which provide the main mechanism of protection against infection. Selenium is a component of glutathione peroxidase enzyme which destroys free radicals in the cytoplasm, whereas vitamin E is a non-enzyme scavenger of free radicals that functions as a specific lipid soluble antioxidant in cell membranes (Noguchi *et al*.,1973; MacPherson, 1994 and Smith *et al*., 1997).

A diet deficient in vitamin E may increase the amount of selenium needed to prevent certain abnormalities, such as nutritional muscular dystrophy because while there is a sparing influence on a deficiency of the other, neither can fully compensate for the absence of the other nutrient. The complementary functions of selenium and vitamin E have been hypothesized to suggest that supplementation with one can reduce, but not eliminate the requirement for the other (Maas, 1983; Miller *et al*., 1988).

Alpha-tocopherol is present in the lipid bilayers of biological membranes where it may play a structural role. Polyunsaturated fatty acids (PUFA) of membranes are particularly vulnerable to attack by reactive oxygen species, and reactive oxygen species can initiate a chain reaction of lipid destruction that destroys the membrane of the cell. Vitamin E can quench peroxidation reactions in membranes and is probably the most important antioxidant located in cell membranes (Putnam and Comber, 1987 and Gurr and Harved, 1991).

There are several reasons why extensive lipid peroxidation might prove particularly damaging to immune response. First ,cells of the immune system are highly dependent on a functioning cell membrane. In addition to normal metabolic activity occurring at the membrane level ,immune phenomena requiring membrane activity include secretion of lymphokines and antibodies, antigen reception, lymphocyte transformation, and contact cell lysis (Strom, *et al*.1973).

2-4-Deficiency of selenium and vitamin E:

Several diseases of farm animals are caused by or associated with deficiency of selenium and/or vitamin E. Nutritional muscular dystrophy is one of these diseases which is of economic importance particularly in domestic ruminants (Gallo-Torres, 1972 and Hadlow, 1974).

The selenium-and vitamin E-responsive or deficiency diseases of farm animals are caused by diets deficient in selenium and/or vitamin E, with or without the presence of conditioning factors such as an excessive quantity of polyunsaturated fatty acids in the diet. Almost all of the diseases that occur naturally have been reproduced experimentally using diets deficient in selenium and/or vitamin E. Vitamin E deficiency occurs most commonly when animals are fed inferior quality hay or straw or root crops (Radostits *et al*., 2007).

It is generally held that although enzootic muscular dystrophy can result from a dietary deficiency of selenium or vitamin E, precipitating factors such as unaccustomed muscular activity, exposure to other dietary or climatic "stress," intercurrent disease, or myopathic agents in the feed (unsaturated fatty acids in fish and vegetable oils) may convert an asymptomatic deficiency state to one of frank disease of the musculature (Blood and Henderson, 1968).

Inadequate vitamin E intake has led to myopathic conditions in animals ingesting high levels of poly-unsaturated fatty acids (PUFA), probably due to insufficient vitamins to prevent autoxidation of fatty acids within tissues. For the experimental production of muscular dystrophy in lambs and calves, large amounts of unsaturated fats have been considered a predisposing factors for the production of the condition (Erwin *et al.*, 1961).

It should be noted that sources of peroxides within the cells have been identified which correlate well with the destruction of phospholipid polyunsaturated fatty acids (PUFA).Vitamin E has been shown to inhibit this destruction (Tarn and McCay, 1970).

Feeding cod liver oil to ewes during pregnancy and lactation was an effective means of producing muscular dystrophy in lambs. The most effective method of administration of the oil was a combination of the oil with a ground corn and corn cob mixture. The cod liver oil lowered ewe and lamb blood plasma concentrations of vitamin E, and this reduction was associated with the incidence of dystrophy (Welch *et al*.,1960).

Deficiencies occur when animals are fed poor quality hay or straw and lack access to pasture. Diets high in polyunsaturated fatty acids contribute to the development of nutritional muscular dystrophy by increasing the requirement for vitamin E (Pugh, 2002).

Since the need for selenium is related to the dietary supply of vitamin E, animals raised under intensive management in selenium-deficient areas frequently exhibit deficiency signs when not supplemented with selenium and vitamin E (NRC, 1971).

Selenium deficiency is typically more prevalent in areas of high rainfall than drier regions, probably due to leaching of selenium from the pastures. Selenium uptake by crops and forages is also influenced by soil pH, being higher on alkaline than acid soils. In addition, selenium deficiency can be exacerbated by, or result from, continual and/or intensive farming practices. Vitamin E deficiency is independent of soil type and more closely reflects forage/feed quality. In general terms, fresh legumes and pasture are good sources of vitamin E, whereas silage, oil seeds, root crops, cereal grains and dry hays are not. Vitamin E concentrations are naturally high in green swards but fall rapidly during dry periods, or in stored forages, hay and grain. Prolonged storage of feedstuffs can result in up to 50 per cent losses of vitamin E per month (Aitken, 2007).

In recent years, it has been recognized that selenium deficiency is a much more serious problem among grazing animals than is the toxicity of the element. Nutritional muscular dystrophy, also referred to as white muscle disease, is the major sign of a severe deficiency in ruminants. This occurs primarily in young calves and lambs born to selenium-deficient dams (Maas and Valbery, 2009).

Selenium deficiency in various animal species is manifested by both nonspecific and specific clinical signs. Their severity depends on the degree of deficiency. Well-known animal diseases due to selenium deficiency include nutritional myodystrophy of ruminants, hepatic necrosis of rats, pancreatic degeneration, reproductive failures, encephalomalacia of chicks, and others. Due

to the complementary role of selenium and vitamin E ,all selenium deficiency diseases in animals are concordant with vitamin E deficiency (Shamberger, 1983; Oldfield, 1999; McDowell, 2000 and Leng *et al*., 2003).

Muscular dystrophy associated with selenium and vitamin E deficiencies ranks with frequently occurring disorders described in many animal species, in addition to selenium and vitamin E deficiency, a significant role in the development of the disease is attributed to a high intake of unsaturated fatty acids increasing the concentration of hydroperoxides in the organism (Pavlata, 2001) b. The majority of the reported cases resulted from a combined selenium and vitamin E deficiency (Deger *et al*., 2008).

White muscle disease or nutritional muscular dystrophy is most common in neonatal and fast growing young animals and is caused by deficiency of selenium and vitamin E or both. The prevalence of clinical vitamin E deficiency, without concurrent selenium deficiency, in lambs is not known (Surai, 2006).

Lipid soluble vitamin E is part of the antioxidant system that protects those cellular components high in lipids (such as the cellular membrane, mitochondria, endoplasmic reticulum, and plasma membranes) from lipoperoxidation by decreasing hydroperoxide formation. Selenium, which is a necessary component of glutathione peroxidase (GSH-PX), also acts to reduce oxidative damage by destroying peroxides in the body's more aqueous environment. Diets high in vitamin E, but deficient in selenium, may still result in the development of white muscle disease in lambs, since the nonmembrane proteins are not protected by the glutathione peroxidase (GSH-PX) system and so are at risk of oxidative damage. Conversely, diets deficient in vitamin E, but adequate in selenium, also may put at risk some subcellular components that are

not protected by the glutathione peroxidase (GSH-PX) system (Radostits *et al*., 2000).

Vitamin E deficiency usually occurs in animals that are fed poor quality hay with no other source of vitamin E or diets that are high in polyunsaturated fatty acids (Menzies *et al*., 2004). Experimental vitamin E myopathy has been induced in lambs by adding cod-liver oil to the milk replacer (Steiss,1985). Serum selenium concentration was found to decrease with the advance of pregnancy in sheep (Whiet *et al*., 1989).

Pregnant ewes deficient in vitamin E and/or selenium may increase the incidence of stillborn progeny, or weak lambs, which only survive for a few days before dying because of acute heart failure (Hamliri *et al*., 1990).

Deficiencies in alpha-tocopherol are similar to those of Selenium. Nutritional muscular dystrophy, a muscle degenerative disease in young ruminants with a selenium deficiency, is influenced by vitamin E status (McDowell, 1989).

Vitamin E can protect against many of the symptoms of selenium deficiency and vice versa .These sparing as well as synergistic actions are thought to result from the ability of both tocopherol and selenium-dependent glutathione peroxidase to decrease the production of lipid peroxidation products. A study of animals demonstrated that dietary supplementation with vitamin E or selenium alone was ineffective in preventing a chemically induced mammary cancer. However, supplementation with both micronutrients prevented tumor development (Horvath and C., 1983).

Pritchard and Singh (1960) have postulated a destruction of rat tissue polyunsaturated fatty acids (PUFA) in the absence of vitamin E which they attribute to in vivo lipid peroxidation.

A metabolic interrelationship between selenium and vitamin E is well known, and the metabolism of one of these is influenced by the other (Fischer and Whanger, 1977).

Increased polyunsaturated fatty acids intake has been postulated as a naturally occurring "trigger factor" in the induction of myopathy in calves deficient in both selenium and vitamin E (McMurray and McEldowney, 1977).

Hidiroglou, (1977) reported that serum tocopherol level resulted from feeding hay was lower than that resulted from feeding silage to beef cattle. Cary (1939) found that when rabbits were fed a cow ration consisting of a grain mixture, poor quality hay and small amounts of cod-liver oil, they eventually become paralyzed due to muscular dystrophy.

2-5-Effect of deficiency of selenium and vitamin E on immunity parameters:

2-5-1-Phagocytosis:

Vitamin E supplementation to cows around parturition has been reported to prevent suppression of blood neutrophil and macrophage function during early postparturition period and increases phagocyte activity (Politis *et al*., 1995 and Chew, 1995).

Under stress conditions, increased levels of such compounds as prostaglandins, thromboxanes and leukotrienes by endogenous synthesis or exogenous entry may adversely affect immune cell function (Hadden, 1987).

The protection of cell membranes and other cellular components of immune cells against lipid peroxidation is probably the most important mechanism of vitamin E in the immune response (Bendich, 1990).

Selenium deficiency was associated with decreased intracellular kill by bovine neutrophils, while performance of phagocytes can be improved by

selenium/vitamin E injections this was evident when the percentage of viable polymorphonuclear leukocytes-associated bacteria for the treated group was significantly lower than that of the selenium-deficient group indicating that after 20 minutes of exposure to the bacteria , the polymorphonuclear leukocytes from the selenium-vitamin E injected cows killed the organism better than those from nontreated cows, the treatment included the following: (three cows given 7ml of a solution containing 35 mg of sodium selenite and 350 mg vitamin E 16 days before blood samples were taken and two cows given 20 ml of a solution containing 20 mg of sodium selenite and 1000 mg of vitamin E) (Gyang *et al*., 1984).

Alterations in immunity reported with vitamin E deficiency. Reduced lymphocyte and leukocyte killing power has been shown in humans as well as in experimental animals. Vitamin E supplementation has been reported to enhance both humoral and cell-mediated immunity and to augment the efficacy of phagocytosis in experimental and farm animals and humans (Meydani and Blumberg, 1993).

Free radicals and lipid peroxidation are immunosuppressive and due to its strong lipid-soluble antioxidant activity vitamin E is able to optimize and enhance the immune response. Supplementation with vitamin E increases lymphocyte proliferation in response to mitogens , phagocytic activity by alveolar macrophages, and causes an increased resistance against infectious agents (Meydani *et al*., 2005).

Selenium deficiency in goats resulted in decreased polymorphonuclear leucocyte function due to lower glutathione peroxidase activity this was clear when phagocytic functions of polymorphonuclear leukocytes isolated from goats in week 16 of feeding selenium-deficient diet were ,however, severely depressed

compared with those of polymorphonuclear leukocytes from goats given the selenium-adequate diet and functionally depressed polymorphonuclear leukocytes to which selenium was added markedly enhanced their chemotactic and phagocytic functions, peak activities occurred mainly at a concentration of 0.347 µg of selenium/2.5×10^6 cells (Azizi *et al*., 1984).

Boyne and Arthur (1986) mentioned that more contemporary immune-response measures involve abilities of polymorphonuclear cells to phagocytize (engulf) and (or) kill invading organisms and they proved that fact when they concluded from their results that the ability of nutrophil from male and female mice to kill ingested cells of *candida albicans* was lower in selenium –deficient than in selenium-supplemented animals.

The ability of peripheral blood polymorphonuclear to engulf yeast cells, in vitro, was impaired by both vitamin E and selenium deficiencies and was impaired sooner by the combined vitamin E and selenium deficiency than by individual deficiencies of vitamin E or Selenium (Wuryastuti *et al*., 1993).

Vitamin E and selenium are critical antioxidants. Cattle consuming feed low in vitamin E and selenium have increased intramammary infections (Max *et al*., 2003).

The ability to kill ingested *Candida albicans* and *Staphylococcus aureus* was lower in neutrophils isolated from selenium deficient cattle than in selenium adequate controls (Boyne and Arthur, 1979).

It has been suggested by Baumgartner (1979) that vitamin E and selenium modulate the immune response by protecting lymphocytes from the effects of various inhibitory products produced by phagocytes. These products, produced especially by macrophages, can include lipid hydroperoxides, superoxide anion, singlet oxygen, hydrogen peroxide, hydroxyl radical and prostaglandins. In

addition to the deleterious effect of these reactive oxygen species, the immune system can be severely affected by alterations in the integrity of cellular membranes. Cellular membranes are involved in the release of these soluble substances, as well as being of great importance in the binding of mitogens and antigens, and binding and subsequent lysis of foreign cells by various cytotoxic effector cells.

When animals are in stressed or disease state, there is an increased production of glucocorticoids ,epinephrine, eiecosanoids, as well as elevated phagocytic activity which lead to production of free radicals which challenge the animals antioxidant system (Nockles, 1989).

Vitamin E positively influenced neutrophil-antibody dependent cellular cytotoxicity and phagocytosis as well as lymphocyte stimulation in calves fed milk replacer (Pruiett *et al*., 1989). Selenium proved to enhance phagocytic activity in buffalo polymorphonuclear leukocytes starting from parturition up until three weeks post-partum (Ramadan *et al*., 2001).

El-Gaafrawy *et al*., (2000) found that calves aged 3-5 weeks received the additive selenium and alpha-tocopherol acetate, showed blood white cells content and greater phagocytosis than the control.

2-6-Effect of deficiency of selenium and vitamin E on hematology parameters:

2-6-1-Erythrocyte osmotic fragility:

In young cattle from areas where nutritional muscular dystrophy is endemic and particularly at the end of winter housing, the erythrocytes have an increased susceptibility to hemolysis following exposure to hypotonic saline. During clinical and subclinical white muscle disease in calves, there is a significant increase in both the osmotic and the peroxidative hemolysis of the erythrocytes. This defect is thought to be the result of alterations in the integrity of cell membranes of which tocopherols are an essential component. Vitamin E deficiency in sheep results in increased hemolytic susceptibility of erythrocytes, which may provide a basis for a single. functional test for vitamin E deficiency in sheep (Radostits, *et al.* 2007).

Selenium deficiency has been identified as the leading cause of excessive fragility of vascular and erythrocyte membranes, which leads to such condition as anemia with Heinz bodies (Morris *et al*., 1984 and McDowell, 2003).

Heikkila *et al*., (1971) found that during peroxidative hemolysis all phospholipids of the erythrocyte membrane were destroyed in approximately equal ratios. Vitamin E is one of the major lipid soluble antioxidant. It prevents oxidation of polyunsaturated fatty acids and thus protects red blood cells from oxidative stress induced lyses (Chan, *et al.* 1999). Supplementation of vitamin E may have an important role in maintaining red cell membrane integrity by reducing osmotic fragility of erythrocyte (Ono, 1985 and Jaja *et al*., 2005).

Brin *et al*., (1974) have reported that there was an increased osmotic fragility of the red blood cells in a cod liver oil-treated, vitamin E deficient

rabbit. Vitamin E deficiency increases the peroxidative fragility of red blood cells (Horn *et al*., 1974).

Huang *et al*., (1988) reported a significantly elevated red blood cells in vitro hemolysis as a result of lipid peroxidation. The red blood cells in vitro hemolysis test has long been used as a criterion for the assessment of vitamin E status. The higher red blood cells hemolysis implied that the vitamin E status might be compromised by the lipid peroxidation.

Forbes and Draper (1958) have reported that the peroxidative hemolysis of erythrocytes has been used as an index of the vitamin E status.

2-6-2- Total and differential white blood cells count:

Synergism between selenium and vitamin E has been demonstrated by the significant increase in lymphocyte counts in healthy sheep (Larsen *et al*., 1988). Higher total leukocyte counts were observed in animals infected with *haemonchus contortus* and supplemented with selenium , where the neutrophils were responsible for the increase in the total leukocyte count (Emmanuel, 2010).

The total white blood cell count was significantly increased in lambs which become vitamin E deficient by giving 3 ml cod liver oil daily for 62 to 74 days when vitamin E deficiency was complicated with pneumonia (Culik *et al*., 1951).

The increase in the number of lymphocytes in animals supplemented with vitamin E when compared to animals that received only selenium is due to the protection of the lipid membrane, receptors and other cellular components involved in the modulation of the immune response promoted by vitamin E (Meydani , 1995).

Lymphocytes are considered the population of cells most sensitive to free radicals due to the high level of free fatty acids in their membranes (Nemec *et al*., 1990).

Qureshi *et al*., (2001) have reported that vitamin E-selenium supplemented buffaloes had higher eosinophil (3.4% control) (4.8% supplemented) and lymphocyte (63.4% control) (67.6% supplemented) percentages compared with control animals.

Salman, (2003) reported that sheep given oral dose of 400 mg vitamin E per head three times at weekly intervals, viz; before, at and after vaccination with Pox vaccine showed a slight increase in the nutrophils count during the first week and a significant increase in the lymphocytes count along the period of the study.

2-7-Effect of deficiency of selenium and vitamin E on biochemical parameters:

2-7-1-Serum creatine kinase and aspartate aminotransferase:

In mammals, the use of serum creatine kinase has been considered to be the most sensitive and specific enzyme test of myopathic conditions such as occurring in vitamin E and selenium deficiency, this enzyme being relatively specific to striated muscles (Cardinet *et al*., 1967).

Melville and Hummel (1951) demonstrated a significant increase in blood Creatine Kinase with the development of muscular dystrophy.

Relevant to the diagnosis of muscular damage are creatine kinase, aspartate aminotransferase, and lactate dehydrogenase , of which creatine kinase is the most sensitive and specific indicator of muscular damage (Meyer and Harvey, 1998).

Plasma creatine kinase is the most commonly used laboratory aid in the diagnosis of nutritional muscular dystrophy. aspartate aminotransferase activity is also an indicator of muscle damage. The magnitude of the increase in aspartate aminotransferase and creatine kinase is directly proportional to the extent of muscle damage (Radostits, *et al.* 2007).

Diagnosis of white muscle disease relies on necropsy and clinical pathology especially enzymes creatine kinase, aspartate aminotransferase , and lactate dehydrogenase indicative of muscular dystrophy (Bostedt and Schramel, 1990).

Prior to the first clinical signs of white muscle disease in lambs there is a certain increase in blood creatine kinase, aspartate aminotransferase concentrations indicative of muscle degeneration (Chariot and Bignani, 2003).

Elevated plasma activity of the muscle enzyme creatine kinase has been reported in vitamin E and selenium deficient animals (Anderson *et al*., 1977).

Norton and McCarthy, (1986) reported an increase in serum level of creatine kinase in lambs suffering from nutritional muscular dystrophy.

Allen *et al*., (1975) observed that yearling cattle that were exposed to the stress of unaccustomed exercise after confinement and fed hay , straw which were relatively poor sources of vitamin E and propionic acid-stored barely exhibited an increases in creatine kinase activity.

Creatine kinase in plasma has been shown to be elevated in ruminants that exhibit clinical or subclinical nutritional muscular dystrophy, and is highly correlated with the degree of muscle damage in cows (Spears *et al*., 1986).

McMaurry *et al*., (1980) showed that polyunsaturated fatty acids were capable of escaping ruminal hydrogenation at turnout, resulting in a three-fold increase of plasma linolenic acid within three days of turnout.

Rice *et al*., (1981) showed that linolenic acid, if protected from ruminal hydrogenation, rapidly reaches high levels in blood and is associated with a rise in plasma creatine kinase indicating muscular degenerative myopathy. Vitamin E administration to calves suffered from vitamin E deficiency decreased enzymes of muscle origin such as creatine kinase and aspartate aminotransferase (Reddy *et al*., 1987).

Hoshino *et al*., (1989) reported that levels of creatine kinase and aspartate aminotransferase were increased in calves with white muscle disease.

Increased serum levels of the aspartate aminotransferase were proportional to the extent of muscle damage of lambs affected with nutritional muscular dystrophy and this enzyme has been the most commonly used enzyme to indicate muscle damage (Blincoe and Dye, 1958).

Increased serum creatine kinase levels have been recorded in lambs affected by white muscle disease. This enzyme is involved in helping provide energy for muscle contraction. The duration of elevation following muscle injury is considerably less than for aspartate aminotransferase (Cardinet, 1971).

In lambs fed diets lack selenium , aspartate aminotransferase level was markedly elevated after 35 days regardless of clinical symptoms of dystrophy (Erwin *et al*., 1961).

Hopkins *et al*., (1964) and Gardner and Hogue, (1967) reported an increase in serum levels of aspartate aminotransferase as a result of muscle degeneration in lambs with white muscle disease.

The enzyme, aspartate aminotransferase, rose rapidly within two weeks after the lambs were placed on the vitamin E or selenium deficient diet and this rise was indicative of muscle damage, when vitamin E or selenium were supplemented in the diet, the average weekly enzyme levels were significantly decreased (Ewan *et al*., 1968).

Wright and Bell, (1964) and Buchanan-Smith *et al*., (1969) have reported that aspartate aminotransferase activity in plasma increased in vitamin E-deficient ewe lambs, the elevated plasma levels of aspartate aminotransferase and creatine kinase together confirm the presence of necrosis in skeletal muscle.

Whanger *et al*., (1969) have reported an increase in the activity of aspartate aminotransferase in muscle of myopathic lambs.

The increased levels of aspartate aminotransferase, which appears to be proportional to the degree of muscle fiber degeneration , has been used in the diagnosis of nutritional muscular dystrophy in ruminants (Kuttler *et al*., 1961).

In certain cases increased levels of aspartate aminotransferase as reported by Hidiroglout *et al*., (1967) only indicate a case of subclinical nutritional

myopathy which is considered an early diagnostic tool since this increase did not accompany any clinical signs that are indicative of clinical nutritional myopathy. Wastell *et al*., (1972) have reported that serum aspartate aminotransferase activity was high in pigs deficient in vitamin E and selenium.

2-8-Effect of deficiency of selenium and vitamin E on udder health indicators:

2-8-1-Somatic cell count:

Glutathione peroxidase and alpha-tocopherol are important components of the cellular defense system. Together they function to protect the cell membrane, and cell content from oxidative damage. One example of oxidative damage is the reaction of white blood cells to invasion of bacteria into the udder. When bacteria gain entrance to the udder, an influx of white blood cells occurs to fight off the infection. Peroxides (and other substances) are produced by the white blood cells to help destroy the pathogens. Uncontrolled, these peroxides can be dangerous to healthy cells and tissue. Vitamin E and selenium are necessary to help the white blood cells reduce the peroxides to safe substances and to continue destruction of invading pathogens. When an animal is deficient in vitamin E and/or selenium, this function of white blood cells is impaired (Craven and Williams, 1985).

Erskine *et al*., (1989) found considerably lower concentrations of selenium in herds with high somatic cell count (>700,000) as compared to herds with low somatic cell count (<150,000). Dietary supplementation of selenium to first lactation heifers at (0.3 mg/kg of DM) resulted in significantly lower somatic cell counts (Smith *et al*., 1987).

In at least two field studies that examined relationships among dietary and serum selenium, bulk tank somatic cell counts , and clinical mastitis, high

concentrations of serum selenium were associated with reduced rates of clinical mastitis and lower bulk tank somatic cell counts (Weiss *et al*., 1990).

Selenium and vitamin E supplemented heifers had significantly fewer quarters infected at calving, reduced prevalence of infection throughout lactation, fewer cases of clinical mastitis, infections of shorter duration, and lower milk somatic cell counts compared with unsupplemented heifers (Smith *et al*., 1985).

Malbe *et al*., (1995) reported that supplementation of a very low selenium diet with 0.2 ppm of selenium from selenite or selenized yeast reduced somatic cell counts in lactating dairy cows.

Wichtel *et al*., (1994) mentioned that mean somatic cell counts over an entire lactation was reduced or tended to be reduced when cows were supplemented with 6 to 12 mg of selenium/day. Braun *et al*., (1991) reported that very low plasma or blood selenium levels results in high incidence of mastitis or high somatic cell count in cows from farms known to be encountering increased incidence of mastitis.

Dairy herds with low somatic cell counts had significantly higher mean blood glutathione peroxidase (GSHPX) and higher whole blood concentrations of selenium than in herds with high somatic cell counts. The prevalence of infection due to *Streptococcus agalactiae* and *Staphylococcus aureus* was higher in herds with the high somatic cell counts compared with those with the low somatic cell counts. This suggests that phagocytic function in the mammary gland may be decreased by a marginal selenium deficiency (Radostits, *et al.* 2007).Neutrophils from selenium-deficient animals were less capable of intracellular killing of mastitis pathogens (Smith *et al*., 1997).

Chapter Three

Materials and Methods

3-1-Materials:

3-1-1-Reagents, stains and solutions:

- **Chemicals used in erythrocyte osmotic fragility:**

  -Sodium chloride NaCl (absolutely dry)
  -Distilled water

- **Chemicals used in white blood cells total count:**

  -Turkis fluid purchased from local market / Baghdad, Iraq.

- **Chemicals used in white blood cells differential count:**

  -Giemsa stain purchased from local market / Baghdad, Iraq.
  -Absolute methanol

- **Chemicals used in phagocytosis:** (Metcalf *et al.*,1986)

  -Hank's Balanced Salt Solution HBSS:
  Prepared as follows:
  Stock A:

NaCl	16 gm
MgSo4 7H2O	0.4gm
CaCl2	0.28
KCl	0.8
Distal Water	100 ml

  Stock B:

Na2HPO4	0.3gm
KH2PO4	0.12gm
Glucose	2gm

Phenol red	0.2gm
Distal Water	100 ml

Solutions A:B:Distal water were mixed as 1:1:18 volumes and the pH was adjusted to 7.2 ,then autoclaved and stored at 4°C.

- Absolute methanol

- Giemsa stain

Killed yeast suspension : Metcalf *et al*.,(1986)

Fifty grams of dried bread yeast *Saccharomyces cerevisiae* were suspended in 150 ml of sterile normal saline ,and the mixture was boiled in water bath for one hour , then the suspension was filtrated by a double sterile layers of gauze , and the cells number of suspension was enumerated by haemocytometer and it was adjusted to 10^9 cell /ml, finally the suspension was stored by freezing.

- **Chemicals used in Somatic cell count:** (Coles, 1986)

Newman-Lambert stain which is composed of the following:

-Certified powdered methylene blue	1.12gm
-Ethyl alcohol(95%)	54.0ml
-Tetrachloroethane	40.0ml
-Glacial acetic acid	6.0ml

- **Chemicals used in vitamin E estimation:**(Varley, *et al*.,1976)

-Xylene :		GCC	England	99.9%
-2-2-dipyridyl	1.2gm	Merck	Germany	99.9%
-Ferric chloride	1.2	Qualikems	India	99.9%
-Alpha-tocopherol powder	10mg	BDH	England	99.9%
-Propanol absolute	1L	Qualikems	India	99.9%
-Ethanol absolute	2L	Qualikems	India	99.9%

- **Chemicals used in selenium estimation:**(Norheim and Haugen, 1986)

-Selenium standard	Buck Scientific	USA	99.9%
-Perchloric acid	Himedia	India	99.9%
-Nitric acid	GCC	England	99.9%
-Hydrochloric acid	BDH	England	99.9%

-Sodium borohydride	BDH	England	99.9%
-Sodium hydroxide	Banaras	India	99.9%

- **Chemicals used in serum enzymes estimation:**

-Creatine kinase: Kit from (Linear chemicals, S.L./ Spain).

-Aspartate amino transferase: Kit from (Syrbio/Syria).

3-2-Methods:

3-2-1-Experimental design:

The study lasted for 9 months started from 1.3.2011 to 1.12.2011 and involved the following steps:

1.Selection and grouping of animals.

2.Carrying out an estrus synchronization.

3.Induction of vitamin E and selenium deficiency.

4.Sampling and laboratory work.

3-2-2-Animals of the study:

Forty Awassi ewes at State Board of Agricultural Research / Ministry of Agriculture were selected, 20 ewes were taken from a herd of 120 ewes which were virgin (18 months old) and unvaccinated with Rev 1 vaccine , other 20 ewes were selected from a herd of open animals (26 months old) and vaccinated with Rev 1 vaccine.

3-2-3-Ultrasound scanner:

Ultrasound scanner examination performed on all ewes before estrus synchronization by using real time B-mode scanner equipped with a 7.5MHz linear array prostate transducer which shows a rectangular image.The type of the scanner is (ALOKA SSD-500V USA) (equipped with a 7.5MHz linear array prostate transducer ALOKA Co. Ltd., Japan). It was portable ,being mounted on a mobile trolley. The scanner possessed a freeze mode, a magnification or zoom

mode and an image storage memory with recall. Additionally, the scanner was provided with a press control key board.

The transducer was water proof , electrically insulated and connected to the scanner by a flexible insulated and sealed coaxial cable of 2m in length.

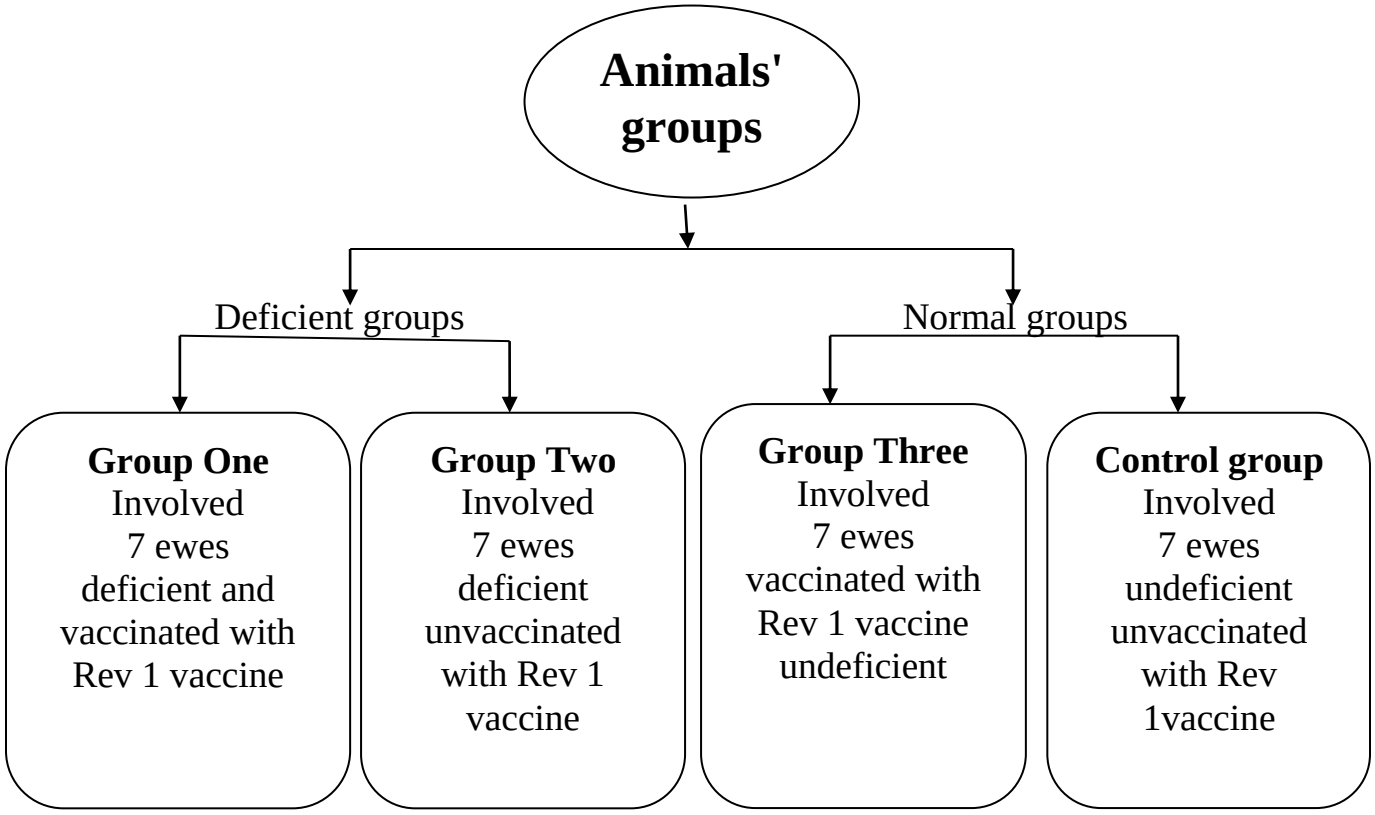
The 40 ewes selected were normal and healthy and were ready to be used for estrus synchronization which was done according to the following schedule:

3-2-4-Estrus synchronization: (Wildeus, 2000)

Estrus synchronization

Group one	**Deficient and vaccinated with Rev1 vaccine**		
Vaginal sponge insertion	Vaginal sponge removal	Mating	Pregnancy testing
19.3.2011	1.4.2011	3.4.2011	22.5.2011
Group Two	**Deficient unvaccinated with Rev1 vaccine**		
Vaginal sponge insertion	Vaginal sponge removal	Mating	Pregnancy testing
7.4.2011	20.4.2011	22.4.2011	22.5.2011
Group Three	**Vaccinated with Rev 1and undeficient**		
Vaginal sponge insertion	Vaginal sponge removal	Mating	Pregnancy testing
29.3.2011	11.4.2011	13.4.2011	22.5.2011
Group Four	**Unvaccinated undeficient**		
Vaginal sponge insertion	Vaginal sponge removal	Mating	Pregnancy testing
13.4.2011	26.4.2011	28.4.2011	22.5.2011

Groups were allocated according to the pregnancy testing results which diagnosed (9 pregnant ewes in the first group), (7 pregnant ewes in the second group),(8 pregnant ewes in the third group) and (7 pregnant ewes in the control group). In order to allocate an equal number of ewes in each group, seven animals were selected and used in the experiment as followed:



3-2-5-Vitamin E and selenium deficient diet:

The diet consisted of the following items: (Welch *et al*.,1960).

1.Ground corn 0.5 kg/animal/day.

2. Cod liver oil 3% (mixed with the ground corn).

3.Hay ad lib.

4.Water ad lib.

At the time of starting feeding vitamin E and selenium deficient diet during the last two months of pregnancy and one month after parturition, the two groups of animals (groups 1 and 2) that were given the deficient diet were placed in two separated pens with closed doors and clean ground provided with consistent water supply and clean mangers. The other two groups (3 and the control group) were allowed the ordinary feeding program of the State Board of Agricultural Research.

3-2-6- Collection of blood samples:

Blood without anticoagulant was collected via jugular venipuncture when clinical signs of the deficiency appeared in ewes and lambs. For serum blood was collected in glass tubes, allowed to stand at room temperature for approximately 30 min, and serum was separated by centrifugation (3000 rpm for 20 min) and then poured into plastic, screw-top disposable centrifuge tubes and stored frozen until estimation of vitamin E and selenium levels and creatine kinase and aspartate aminotransaminase activities (Coles,1986).

Blood with anticoagulant was collected transported at room temperature and each sample evaluated for total and differential white blood cell counts, erythrocyte osmotic fragility and phagocytosis within 2 hours of collection.

3-2-7- Collection of milk and colostrum samples:

Colostrum was collected within the first day of parturition , poured into plastic, screw-top containers and stored frozen at -20°C until use for estimation of vitamin E and selenium. Milk samples were collected after the colostrum days finished (3 days after parturition) then used within 2 hours for performing the somatic cell count, another milk samples were collected and stored frozen at -20°C for estimation of vitamin E and selenium.

3-2-8-Erythrocyte osmotic fragility: (Coles, 1986)

A solution of sodium chloride (1%) was prepared by adding 1gm of sodium chloride to 100 ml volumetric flask containing approximately 40 to 50 ml distilled water. Then sodium chloride was dissolved and brought to 100 ml total volume, using distilled water.

A. Sixteen small test tubes were placed in a rack.

B. A 0.6 ml of saline and 0.4 ml of distilled water were added to the first tube. To the second tube 0.58 ml saline and 0.42 ml distilled water were added decreasing amounts of saline (0.02 ml) were added to each tube until the last tube contains 0.30 % saline.

C. Then blood was drawn to be tested, using a dry syringe, and one drop of blood was added to each tube. Blood with anticoagulant was used , thus the specimen centrifuged, plasma replaced with 0.85 % saline and one drop of the saline suspension of the blood cells was added to the prepared saline tubes.

D. Tubes were allowed to stand at room temperature for two hours.

E. The saline concentration was recorded for beginning hemolysis and complete hemolysis.

3-2-9-White blood cells:- (total and differential counts) were done according to (Coles, 1986).

-Differential white blood cells count:

A thin blood smear was prepared on a clear slide, dried at room temperature , fixed with methanol and stained with 10% Giemsa stain for 10 minutes, then washed with tap water, air dried finally examined with oil immersion. Then the objectives of the microscope were carefully changed, 100 white blood cells were counted and percentage of each type of white blood cells was recorded.

-Total white blood cells count:

Diluting pipettes for a total leukocyte count have two markings that are used in making dilution of blood for counting. These are 0.5 mark on the capillary tube portion of the pipette and 11 mark just over the bulb of the pipette, these marks represent a ratio of 1:20 or a dilution of 0.5 part to 10 parts.

With gentle suction, blood was drawn to the 0.5 mark, the diluting fluid was then drawn to the mark 11 and the blood and the diluting fluid were mixed by shaking the pipette vigorously for two to three minutes.

Then the tip of the pipette was touched to the side of the counting chamber and a drop of fluid was allowed to run under the cover glass that was placed on the chamber.

Counting of the leukocytes started after the cells settled for one to two minutes. Using the low power objective of the microscope ,the corner ruled square was located ,then all the cells in the 16 squares within the larger ruled area in the corner were calculated and the following formula was applied:

Total leukocytes in 4 sq mm multiplied by 50=Leukocytes/µL.

3-2-10-Phagocytosis: (Weber *et al.*,1982)

The phagocytic cell ability for engulfing cells of killed yeast was determined as follows:

An amount of 0.25 ml of heparinized blood from animals of the study was mixed with 0.25 ml of Hank's solution and 0.05 ml of killed yeast suspension in sterile tubes, then incubated at 37ºC/15 - 30 minutes, after that a thick blood smear was prepared on clear slide, dried at room temperature , fixed with methanol and stained with 10% Giemsa stain for 10 minutes ,then washed with tap water , air dried finally examined with oil immersion (200 cells were randomly enumerated).

$$\textbf{Phagocytic index \%} = \frac{\textbf{no. of engulfing cells}}{\textbf{Total no. of phagocytic cells}}$$

3-2-11-Somatic cell count:*

A direct somatic cell count was completed as follows:

A. Milk sample was mixed thoroughly, to disperse the cream throughout the specimen.

B. A pipette was used to spread 0.01 ml of milk over an area of 1 square centimeter.

C. Then the slide dried on a flat surface and stained using Newman-Lampert stain for two minutes, after that the stain was washed by tap water until excess stain has been removed then the slide was air dried and examined under the oil immersion lens.

D. The cells were counted in 30 fields when the number was low, while when the number was high the cells were counted in 50 fields then the mean of

the cells in the counted fields was calculated and multiplied by the microscopic factor which was 400000.

*(U.S. FDA)

3-2-12-Vitamin E in serum, milk, and colostrum by using spectrophotometer: (Varley *et al*., 1976)

Procedure

	Vitamin E standard	Sample	Blank
Sample	-	1.5ml	-
Vitamin E standard	1.5ml	-	-
Blank (Distilled water)	-	-	1.5ml
Absolute ethanol	-	1.5ml	1.5ml
Distilled water	1.5ml	-	-

Xylene	1.5ml	1.5ml	1.5ml

Tubes were sealed , mixed thoroughly and centrifuged 3000xg for 10 minutes. One ml of xylene upper layer was removed from each tube and placed in a clean and dry tube.

2,2-dipyridyl	1ml	1ml	1ml

Tubes were sealed and mixed thoroughly.
Absorbance of the test sample was read against blank at 460nm.

Ferric chloride	0.33ml	0.33ml	0.33ml

Tubes were mixed thoroughly and absorbances of the test sample and standard were read against blank at 520nm.

Calculation

Vitamin E was estimated in sample according to the following equation:

$$\text{Vitamin E conc. (mg/L)} = \frac{(A2 - 0.29\ A1)}{A3} \times \text{standard conc.}$$

A2=Absorbance of test sample at 520nm.
A1= Absorbance of test sample at 460nm.
A3= Absorbance of standard at 520nm.
0.29=(Extinction Coefficient).

3-2-13-Selenium in serum, milk and colostrum by using flameless atomic absorption: (Norheim and Haugen, 1986)

Procedure

Up to 5 ml of biological material was placed in digestion tubes, and 17 ml of a 70+30 mixture of nitric acid and perchloric acid added together with a few alundum granules to prevent bumping. In order to reduce foaming in some types of samples, the tubes were left overnight before digestion. After cooling, the volume of perchloric acid was adjusted to 40 ml. Se^{+6} was reduced to Se^{+4} with hydrochloric acid. Ten ml of 2.4 M HCL was added to each of the tubes containing samples, which were then warmed in the aluminum block at 120℃ for 30 min. After cooling, the solution was diluted by HCl to 25 ml.

The autosampler, hydride generator, and the AA spectrophotometer were operated at the conditions given in the following table:

Autsampler		AA spectrophotometer	
Number of standards	3	Wavelength	196.0nm
Delay time	60 sec	Lamp current	10mA
Rinse time	60 sec	Slit	1.0
Number of replicates	3	Integration time	5 sec
		Background corrector	off
		Double beam made	Yes
Hydride generator			
Flow rate ,sample	6.5 ml/min		
Flow rate ,NaBH4	1ml/min		
Flow rate, HCL	1ml/min		
Nitrogen pressure	3.5kp/cm2		

Sodium borohydride was dissolved in sodium hydroxide sol. to give 0.6 % w/v NaBH in 0.5 % w/v NaOH. The concentration of hydrochloric acid was 10 M, as recommended by the manufacturer.

3-2-14-Serum Glutamic Oxalacetic Transaminase (SGOT). (Aspartate aminotransferase "AST" (Syrbio / Syria):

Principle

Colorimetric determination of Aspartate Aminotransferase based on the following reaction:

$$\text{L-Aspartate + oxoglutarate} \xrightarrow{\text{AST}} \text{Oxalacetate + L.Glutamate}$$

Oxalacetate formed reacts with 2,4 dinitrophenyhydrazine to yield a colored hydrazone that can be measured at 546nm (530-550).

Procedure

Wavelength	546 nm (530-550)
Temperature	37 ºC
Cuvette	1 cm light path

Following tubes were set for each serum (one blank tube was required for each run).

	Reagent blank	Sample
Samlpe	-	0.1ml
Solution 1	0.5ml	0.5ml
Distilled water	0.1ml	-

Tubes were mixed and let to stand exactly 30 minutes at 37ºC

Solution2	0.5ml	0.5ml

Tubes were mixed and let to stand exactly 20 minutes at 20 to 25℃

NaOH 0.4Normaly	5ml	5ml

Tubes were mixed by gentle inversion. Absorbances at (546nm) were read against blank tube after 5 minutes.

Calculation

Obtain the activity of AST in the serum from the table:

Absorbance	**U/L**	**Absorbance**	**U/L**
0.020	7.0	0.100	36
0.030	10	0.110	41
0.040	13	0.120	47
0.050	16	0.130	52
0.060	19	0.140	59
0.070	23	0.150	67
0.080	27	0.160	76
0.090	31	0.170	89

3-2-15-Serum Creatine Kinase "CK"(Linear cheimicals, S.L./ Spain).

Principle

Creatine kinase catalyzes the reaction between creatine phosphate (CP) and adenosine 5´-diphosphate (ADP) with formation of creatine and adenosine 5´-triphosphate (ATP). The latter phosphorylates glucose to glucose-6-phosphate (G6P) in the presence of hexoquinase (HK). Glucose-6-phosphate is oxidized to Gluconate-6P in the presence of reduced nicotinamide-adenine dinucleotide phosphate (NADP) in a reaction catalyzed by glucose-6-phosphate dehydrogenase (G6P-DH).

The conversion is monitored kinetically at 340 nm by the rate of increase in absorbance resulting from the reduction of NADP to NADPH proportional to the activity of CK present in the sample.

In this test, the presence of N-acetilcysteine (NAC) allows the optimal activation of the enzyme.

$$CP + ADP \xrightarrow[pH\ 6.5]{CK\ (AMP,\ NAC)} Creatine + ATP$$

$$ATP + Glucose \xrightarrow{HK} ADP + G6P$$

$$G6P + (NADP^+) + H_2O \xrightarrow{G6P\text{-}DH} Gluconate\text{-}6P + NADPH + H^+$$

Procedure

1. Working reagent ,samples and controls were preincubated to reaction temperature.

2. The spectrophotometer was set to zero absorbance with distilled water.

3. Into a cuvette the followings were pipette:

Reaction temperature	25ºC
Working reagent	1.0ml
Sample	40µl

4. Tubes containing working reagent and samples were mixed gently by inversion, cuvette was inserted into the cell holder and stopwatch started.

5. Tubes were incubated for 3 minutes and initial absorbance reading was recorded.

6. The absorbance reading was repeated exactly after 1, 2, and 3 minutes.

7. The difference between absorbances was calculated.

8. The mean of the results was calculated to obtain the average change in absorbance per minute ($\triangle$A/min.).

Calculation

$\triangle A/min \times 4127 = U/L$

3-2-16-Statistical analysis:

Statistical analysis of the experimental results was conducted according to SPSS version 13.00 where one and two way ANOVA was used to assess the significance of changes between the control and treated animals. The data was expressed as Mean± Standard Errors (SE) and A P-value<0.05 was considered statistically significant. LSD was carried out to test the significance levels among means of treatments (SPSS, 1996).

Chapter four

Results and discussion

4-1-Relationship between clinical signs, selenium and vitamin E levels:

Lambs born to ewes of groups (1 and 2) showed clinical signs of the deficiency within the first three days of life (Two months after feeding the deficient diet to their dams).

Blood samples which were collected from lambs of groups (1 and 2) after being fed the first colostrum showed lower serum selenium levels that reached (0.02 ± 0.00 and 0.01 ± 0.00 ppm respectively) than that in lambs born to ewes of groups (3 and the control group) which reached (0.40 ± 0.03 and 0.45 ± 0.03 ppm respectively) and lower serum vitamin E levels in lambs of groups (1 and 2) which reached (0.60 ± 0.08 and 0.34 ± 0.05 mg/L respectively) than that of groups (3 and the control group) (2.27 ± 0.22 and 2.45 ± 0.16 mg/L respectively) table 4-1.

Serum selenium and vitamin E levels of newborn lambs showed significant differences between deficient groups (1 and 2) and those of undeficient groups (3 and 4) ($P<0.05$) table 4-1.

The clinical signs of the deficiency in this study were mainly sudden death in 4 lambs out of 14 lambs borne to deficient dams (Figures 1 and 2), these results are in agreement with Hamliri *et al*., (1990) and Radostits *et al*. (2007) who mentioned that animals affected with vitamin E and selenium deficiency may be found in sternal recumbency and unable to stand, collapse and die suddenly without any premonitory signs within a few days.

The expected cause of the death was the acute cardiac arrest and this is in agreement with Hamliri *et al*., (1990) who reported that pregnant ewes deficient in vitamin E and/or selenium may increase the incidence of stillborn progeny, or weak lambs, which only survive for a few days before dying because of acute heart failure.

Other 10 lambs born to deficient ewes which remained live showed variable signs included inability to suckle (Figure 3) ,arched back (Figure 4), weakness, dullness, emaciation and recumbency (Figure 5).

The postmortem changes in the dead lambs were also recorded and were mainly paleness and atrophy of thigh muscle and paleness of heart muscle (Figure 6) and (Figure 7) these results are in concert with those of Menzies *et al*., (2004) who reported that the main presenting complaints were sudden death, as well as ill-thrift, weakness, and painful ambulation in many of the lambs, gross findings included generalized atrophy of skeletal muscles, pallor and occasional white streaking of skeletal muscles. Lambs which survived the deficiency were fed from healthy ewes to keep them healthy and prevent the deficiency they encountered.

In ewes of groups (1 and 2) the clinical signs of the deficiency were apparent almost in all animals and appeared three months after the ewes started to be fed the deficient diet. Blood samples which were collected from ewes of groups (1 and 2) at the day of parturition showed lower serum selenium levels (0.03 ± 0.00 and 0.02 ± 0.00 ppm respectively) than that in ewes of groups (3 and the control group) (0.42 ± 0.02 and 0.47 ± 0.30 ppm respectively) table 4-2 also lower serum vitamin E levels that reached (0.71 ± 0.03 and 0.61 ± 0.09 mg/L respectively) than that in ewes of groups (3 and the control group) which reached (2.78 ± 0.19 and 2.72 ± 0.19 mg/L respectively) table 4-3.

The clinical signs of deficient ewes included loss of body weight, decreased milk production and loss of wool (Figures 8, 9 and 10).Weakness, dullness and recumbency were also found (Figures 11 and 12), these results agree with Pugh, (2002); Aitken, (2007) and Radostits *et al*., (2007) who reported that animals affected with nutritional muscular dystrophy with involvement of cardiac muscle showed acute signs that include recumbency, respiratory distress, and death, while in skeletal muscle involvement hunched appearance, stiff gait, and overall poor production are a predominant clinical manifestations and some adult animals continue to eat, but others are dysphagic because of involvement of the tongue.

Serum selenium and vitamin E levels of ewes showed significant differences between groups (1 and 2) and groups (3 and the control group) ($P<0.05$) table 4-2 and table 4-3.

It was noticed from the results of this study that selenium and vitamin E levels in deficient animals were lower than that in healthy animals ,these results are agreed with Menzies *et al*., (2004) ;Radostits *et al*., (2007) and Panousis *et al*., (2007) they reported that tocopherol levels in the serum of less than 2 mg/L in cattle and sheep are considered to be critical levels below which deficiency diseases may occur, and in the early stages of the subclinical form of nutritional muscular dystrophy (NMD) in lambs, there may be a decrease in serum selenium.

The decreased levels of vitamin E and selenium in serum of deficient animals can be explained according to the fact that there is a positive correlation between the selenium and vitamin E content of feed and the selenium and vitamin E content of the tissues and blood of animals ingesting that feed and the values fluctuate with the dietary intake of the element (Radostits *et al*., 2007).

The results of this study are also in agreement with Demirel *et al*., (2004) and Judith *et al*., (2005) who reported that supplementing ewes with long-chain polyunsaturated fatty acids (PUFA) (fish oil) from 6 weeks prepartum until 4 weeks postpartum resulted in a reduction in plasma vitamin E concentrations in both ewes and suckling lambs and a reduction in activity of glutathione peroxidase in suckling lambs.

Milk samples which were collected after the colostrum days ended (3 days after parturition) from ewes of groups (1 and 2) showed lower selenium levels that reached (0.02 ± 0.00 and 0.01 ± 0.00 ppm respectively) than that in ewes of groups (3 and the control group) (0.36 ± 0.02 and 0.40 ± 0.02 ppm respectively). These results showed significant differences between groups (1, 2) and groups (3 and the control group) ($P<0.05$) table 4-2.

Colostrum samples which were collected at parturition from ewes of groups (1 and 2) showed lower selenium levels (0.01 ± 0.00 and 0.01 ± 0.00 ppm respectively) than that in ewes of groups (3 and the control group) (0.31 ± 0.01 and 0.35 ± 0.02 ppm respectively).

These results showed significant differences between groups (1, 2) and groups (3 and the control group) ($P<0.05$) table 4-2.

The results of this study revealed that there was an apparent relationship between maternal and neonatal selenium status, these results are agreed with Quigley and Drewry, (1998) who reported that proper selenium supplementation of the dry cow prior to calving and ingestion of colostrum by calves is critical to providing sufficient selenium to neonatal calves. Weiss *et al*., (1984) reported that the prepartum selenium supplementation of the dam elevated selenium of blood serum in the calf at birth.

Milk samples which were collected after the colostrum days ended (3 days after parturition) from ewes of groups (1and 2) showed lower vitamin E levels (0.48 ± 0.05 and 0.52 ± 0.05 mg/L respectively) than that in ewes of groups (3 and the control group) (1.64 ± 0.13 and 1.62 ± 0.14 mg/L respectively). These results showed significant differences between groups (1, 2) and groups (3 and the control group) ($P<0.05$) table 4-3.

Colostrum samples which were collected at parturition from ewes of groups (1and 2) showed lower vitamin E levels (0.62 ± 0.04 and 0.52 ± 0.04 mg/L respectively) than that in ewes of groups (3 and the control group) (2.11 ± 0.15 and 2.04 ± 0.14 mg/L respectively).

These results showed significant differences between groups (1, 2) and groups (3 and the control group) ($P<0.05$) table 4-3.

It is clear from the results that vitamin E levels in milk and colostrum were lower in deficient ewes than that in healthy ewes, these results are in agreement with those of Judith *et al*., (2005) who reported that supplementing ewes with long-chain polyunsaturated fatty acids (PUFA) (fish oil) from 6 weeks prepartum until 4 weeks postpartum resulted in a reduction in vitamin E concentrations in colostrum and milk.

The results of this study are also in line with Quigley and Drewry, (1998) who reported that colostrum from cows that are not supplemented with vitamin E during the dry period may provide inadequate vitamin E to calves after birth.

Figure (1) Dead lamb in group (1)

Figure (2) Dead lamb in group (2)

Figure (3) Lamb which was unable to suckle in group (1)

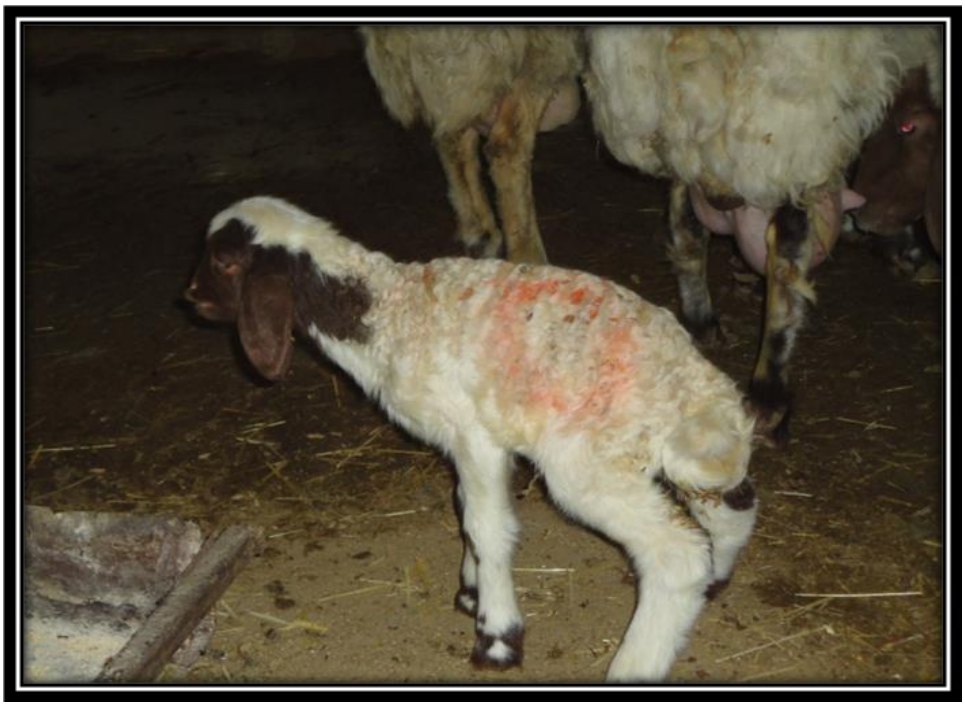


Figure (4) An arched back of lamb in group (2)

Figure (5) Lamb with dullness and recumbency in group (1)

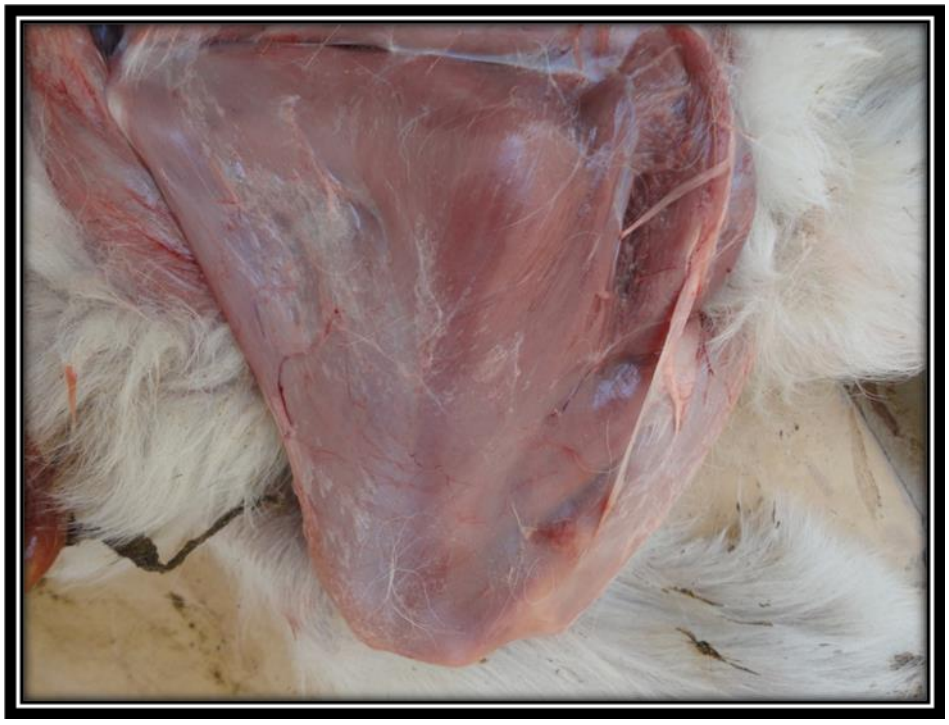


Figure (6) Paleness and atrophy of thigh muscle of a dead lamb in group (1)

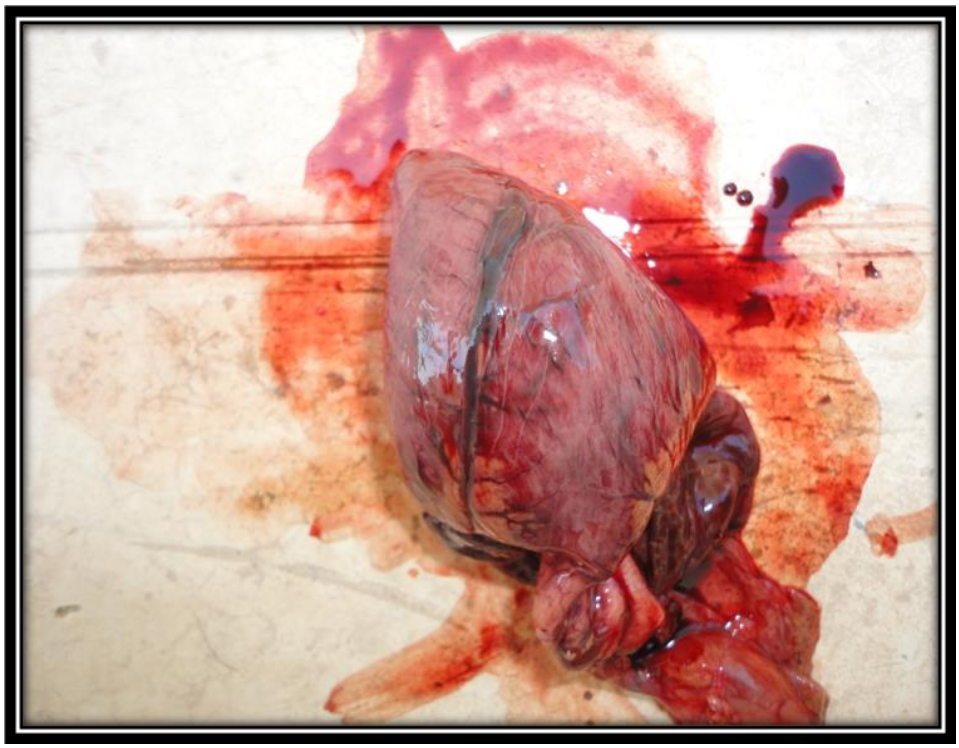


Figure (7) Paleness of heart muscle of a dead lamb in group (1)

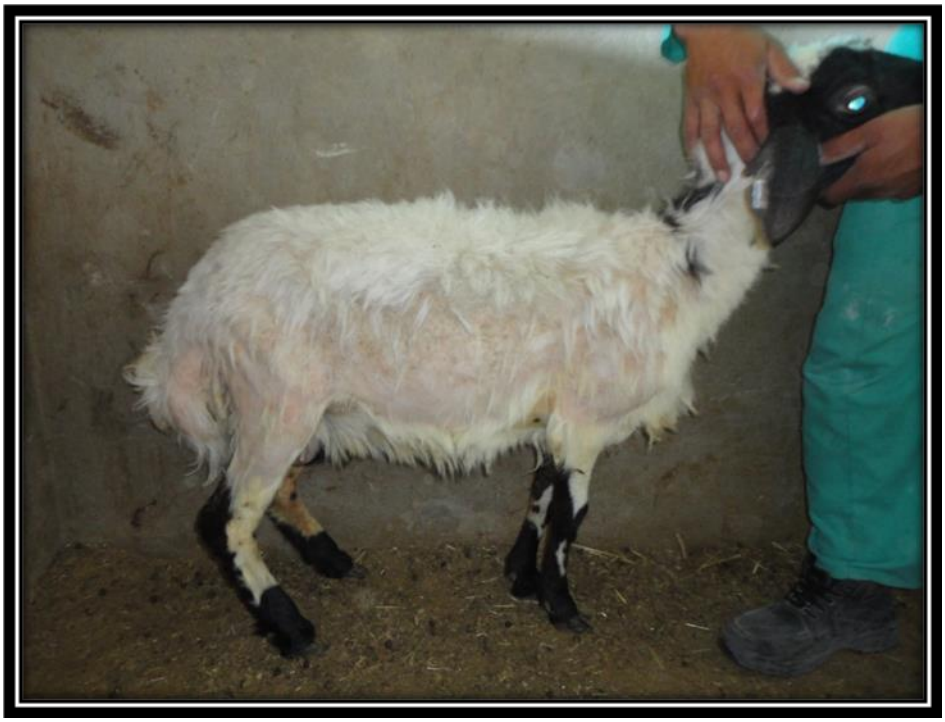


Figure (8) A ewe showed loss of body weight and wool in group (1)

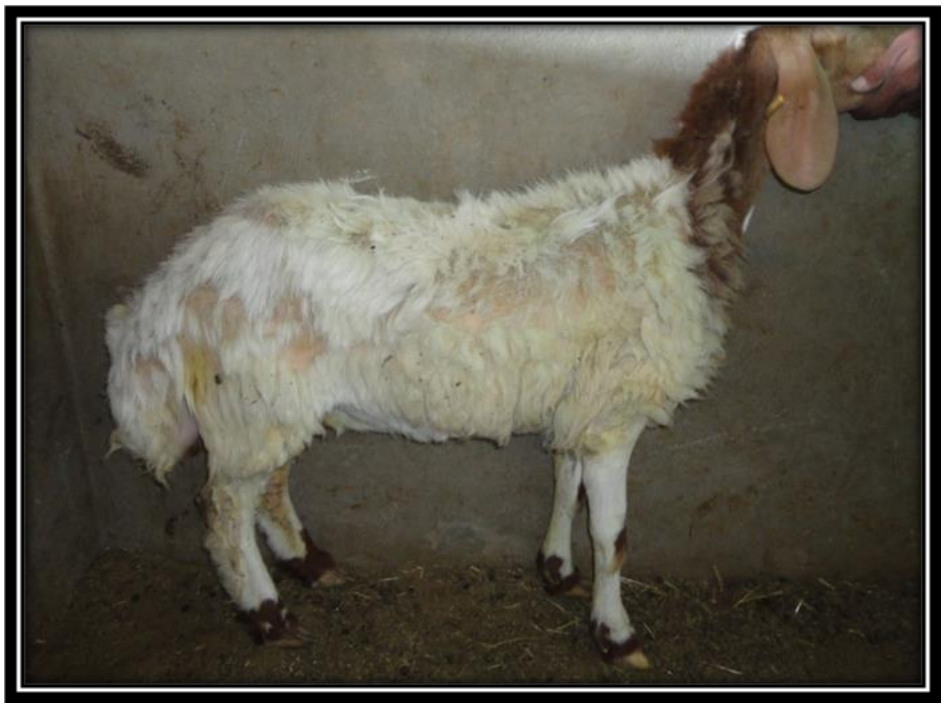


Figure (9) Loss of body weight and loss of wool in a ewe in group (2)

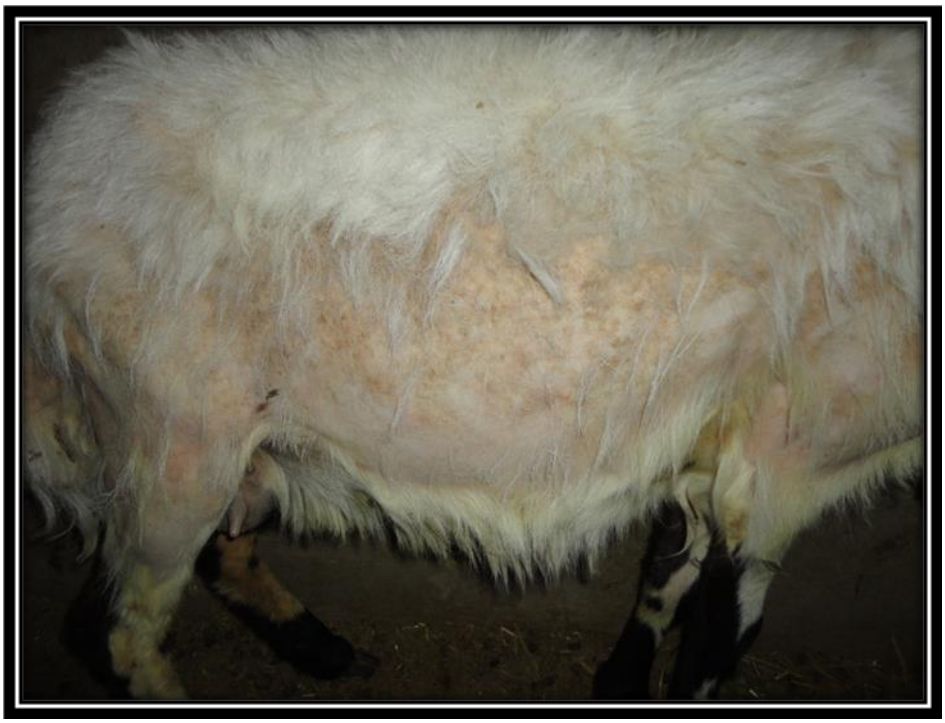


Figure (10) Loss of body weight and loss of wool in a ewe in group (2)

Figure (11) Weakness, dullness and recumbency in a ewe in group (1)

Figure (12) Weakness, dullness and recumbency in a ewe in group (1)

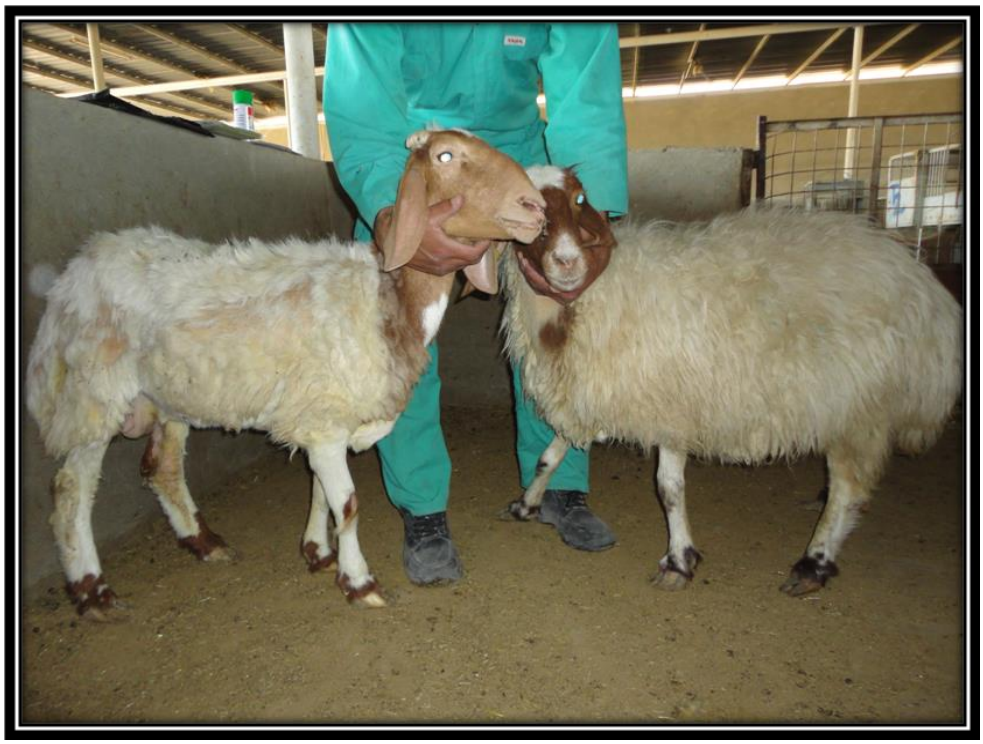


Figure (13) Healthy and deficient ewe

Figure (14) Healthy and deficient ewe

Table(4-1) Selenium and vitamin E levels in serum of newborn lambs

Parameters / Groups	Serum selenium mean ±S.E (ppm)	Serum vitamin E mean ±S.E (mg/L)
Group 1* Born to deficient and vaccinated ewes	0.02 ± 0.00 A	0.60 ± 0.08 A
Group 2* Born to deficient ewes	0.01 ± 0.00 A	0.34 ± 0.05 A
Group 3 Born to vaccinated ewes	0.40 ± 0.03 B	2.27 ± 0.22 B
Control group Born to control ewes	0.45 ± 0.03 B	2.45 ± 0.16 B

n=7

*Tow lambs died

Different letters mean significant (P<0.05) results between different groups

Table(4-2) Selenium levels in serum, milk and colostrum of different groups of ewes

Parameters / Groups	Serum selenium mean ±S.E (ppm)	Milk selenium mean ±S.E (ppm)	Colostrum selenium mean ±S.E (ppm)
Group 1 Deficient and vaccinated ewes	0.03 ± 0.00 A	0.02 ± 0.00 A	0.01 ± 0.00 A
Group 2 Deficient ewes	0.02 ± 0.00 A	0.01 ± 0.00 A	0.01 ± 0.00 A
Group 3 Vaccinated ewes	0.42 ± 0.02 B	0.36 ± 0.02 B	0.31 ± 0.01 B
Control group	0.47 ± 0.30 B	0.40 ± 0.02 B	0.35 ± 0.02 B

n=7

Different letters mean significant (P<0.05) results between different groups

Table(4-3) vitamin E levels in serum, milk and colostrum of different groups of ewes

Parameters / Groups	Serum vitamin E mean ±S.E (mg/L)	Milk vitamin E mean ±S.E (mg/L)	Colostrum vitamin E mean ±S.E (mg/L)
Group 1 Deficient and vaccinated ewes	0.71 ± 0.03 A	0.48 ± 0.05 A	0.62 ± 0.04 A
Group 2 Deficient ewes	0.61 ± 0.09 A	0.52 ± 0.05 A	0.52 ± 0.04 A
Group 3 Vaccinated ewes	2.78 ± 0.19 B	1.64 ± 0.13 B	2.11 ± 0.15 B
Control group	2.72 ± 0.19 B	1.62 ± 0.14 B	2.04 ± 0.14 B

n=7

Different letters mean significant (P<0.05) results between different groups

4-2-Assessment of phagocytic activity:

The results showed a lower phagocytic index in lambs born to ewes of groups (1 and 2) with means that reached (9.40 ± 0.87 and 10.60 ± 1.16 respectively) than that of lambs of groups (3 and the control group) in which the means reached (41.00 ± 0.69 and 43.85 ± 0.34 respectively) table 4-4.Number of phagocytic cells which were engulfing yeast cells in deficient animals ranged between 20 to 25 and in healthy animals ranged between 45 to 50.

The results showed a significant difference in phagocytic index (P<0.05) between groups (1, 2) and groups (3 and the control group) table 4-4.The results also showed a significant lower phagocytic index in ewes of groups (1 and 2) with means that reached (12.14 ± 0.85 and 12.42 ± 0.75 respectively) than that of ewes of groups (3 and the control group) in which the means reached (42.28 ± 0.80 and 43.14 ± 0.91 respectively).The results showed a significant difference

($P<0.05$) between groups (1 and 2) and groups (3 and the control group) table 4-7.

Phagocytosis was depressed in deficient animals of this study, this is in agreement with Wuryastuti, *et al*. (1993) who mentioned that the ability of peripheral blood polymorphonuclear to engulf yeast cells, in vitro, was impaired by both vitamin E and selenium deficiencies and was impaired sooner by the combined vitamin E and selenium deficiencies than by individual deficiencies of vitamin E or selenium.

Immune cells such as the neutrophil, macrophage and other cells are prone to be affected by oxidative stress which can be prevented by vitamin E. Bendich, (1990) mentioned that the protection of cell membranes and other cellular components of immune cells against lipid peroxidation is probably the most important mechanism of vitamin E in the immune response. Furthermore, Gyang *et al*., (1984) reported that selenium deficiency was associated with decreased intracellular kill by bovine neutrophils, while performance of phagocytes can be improved by selenium and vitamin E injections.

The results of this study are consistent with that of Sang-Huan *et al*., (1982) who reported that peritonial macrophages of rats fed vitamin E and selenium deficient diet that contains in its ingredients 3% cod liver oil had significantly lower survival rate during phagocyotosis and when rats supplemented with these two nutrients showed a higher phagocytic activity of their phagocytes, vitamin E prevented lipid peroxidation in macrophage membrane probably through the interaction with the lipid membrane of cells, but selenium functions by destroying hydrogen peroxide (H_2O_2) produced during phagocytosis thereby protecting the membrane from oxidative damage.

The results in this study are also agreed with Boyne and Arthur, (1979) who reported that the ability to kill ingested *Candida albicans* and *Staphylococcus aureus* was lower in neutrophils isolated from selenium deficient cattle than in selenium adequate controls. An evidence that vitamin E and selenium have an improving effect on phagocytic activity in sheep was presented by Milad *et al*., (2001) who reported that supplemented sheep with vitamin E and selenium had an increased phagocytic activity while a deficiency in selenium in ruminants has been shown to be associated with impaired killing ability of phagocytic neutrophils (NRC, 2001).

Table (4-4) The mean percentage of phagocytic activity in newborn lambs

Groups	Phagocytic index percentage mean ±S.E
Group 1* Born to deficient and vaccinated ewes	9.40 ± 0.87 A
Group 2* Born to deficient ewes	10.60 ± 1.16 A
Group 3 Born to vaccinated ewes	41.00 ± 0.69 B
Control group Born to control ewes	43.85 ± 0.34 C

n=7

*Two lambs died

Different letters mean significant (P<0.05) results between different group

Table (4-5) The mean percentage of phagocytic activity in ewes

Groups	Phagocytic index percentage mean ±S.E
Group 1 Deficient and vaccinated ewes	12.14 ± 0.85 A
Group 2 Deficient ewes	12.42 ± 0.75 A
Group 3 Vaccinated ewes	42.28 ± 0.80 B
Control group	43.14 ± 0.91 B

n=7

Different letters mean significant (P<0.05) results between different groups

4-3-Osmotic fragility:

The results of osmotic fragility showed that there was a significant increase in the means of start hemolysis in lambs of groups (1 and 2) that reached (0.54 ± 0.01 and 0.52 ± 0.01 respectively) in comparison to means of lambs of groups (3 and the control group) which reached (0.44 ± 0.00 and 0.42 ± 0.01 respectively) while means of complete hemolysis in lambs of groups (1 and 2) were (0.44 ± 0.00 and 0.42 ± 0.01 respectively) as compared with the means of lambs of groups (3 and the control group) which were (0.34 ± 0.01 and 0.34 ± 0.01 respectively).The results showed a significant difference (P<0.05) in start and complete hemolysis between groups (1, 2) and groups (3 and the control group) table 4-6.

The results showed higher osmotic fragility of erythrocytes of ewes in groups (1 and 2) (0.53 ± 0.00 and 0.54 ± 0.01 respectively) than that of ewes of groups (3 and the control group) in which the means reached (0.44 ± 0.01 and 0.43 ± 0.00 respectively) and higher means of complete hemolysis in ewes of

groups (1 and 2) which were (0.43 ± 0.00 and 0.44 ± 0.01 respectively) than those of ewes in groups (3 and the control group) which reached (0.32 ± 0.00 and 0.33 ± 0.01 respectively).

The results showed a significant difference ($P<0.05$) in start and complete hemolysis between groups (1, 2) and groups (3 and the control group) table 4-7.

The results of this study indicated that in deficient animals the erythrocyte osmotic fragility was high, this agrees with Radostits *et al*., (2007) who reported that vitamin E deficiency in sheep results in increased hemolytic susceptibility of erythrocytes, which may provide a basis for a single functional test for vitamin E deficiency in sheep. Furthermore Morris *et al*., (1984) and McDowell, (2003) reported that selenium deficiency has been identified as the leading cause of excessive fragility of vascular and erythrocyte membranes, which leads to such condition as anemia with Heinz bodies.

The increased hemolysis of erythrocytes in vitamin E and selenium deficient animals was expected to be caused by rapid destruction of erythrocytes cell membrane this was in line with Heikkila *et al*., (1971) who found that during peroxidative hemolysis all phospholipids of the erythrocyte membrane were destroyed in approximately equal ratios.

The fact that vitamin E protects the red cell membrane from oxidative destruction is in concert with Ono, (1985) and Jaja *et al*., (2005) who mentioned that supplementation of vitamin E may have an important role in maintaining red cell membrane integrity by reducing osmotic fragility of erythrocyte.

In addition Chan *et al*., (1999) mentioned that vitamin E prevents oxidation of polyunsaturated fatty acids and thus protects red blood cells from oxidative stress induced lyses.

Table (4-6) Start and complete erythrocyte osmotic fragility in newborn lambs

Groups	Osmotic fragility mean ±S.E	
	(Start hemolysis)	(Complete hemolysis)
Group 1* Born to deficient and vaccinated ewes	0.54 ± 0.01 A	0.44 ± 0.00 A
Group 2* Born to deficient ewes	0.52 ± 0.01 A	0.42 ± 0.01 A
Group 3 Born to vaccinated ewes	0.44 ± 0.00 B	0.34 ± 0.01 B
Control group Born to control ewes	0.42 ± 0.01 B	0.34 ± 0.01 B

n=7

*Two lambs died

Different letters mean significant (P<0.05) results between different groups

Table (4-7) Start and complete erythrocyte osmotic fragility in ewes

Groups	Osmotic fragility mean ±S.E	
	(Start hemolysis)	(Complete hemolysis)
Group 1 Deficient and vaccinated ewes	0.53 ± 0.00 A	0.43 ± 0.00 A
Group 2 Deficient ewes	0.54 ± 0.01 A	0.44 ± 0.01 A
Group 3 Vaccinated ewes	0.44 ± 0.01 B	0.32 ± 0.00 B
Control group	0.43 ± 0.00 B	0.33 ± 0.01 B

n=7

Different letters mean significant (P<0.05) results between different groups

4-4-Total and differential white blood cell counts:

Lambs born to ewes of groups (1 and 2) showed significant lower white blood cell count with means that reached (2.88 ± 0.12 and 2.88 ± 0.18 x 10^3 / µl respectively) as compared with the means of lambs born to ewes of groups (3 and the control group) which reached (9.07 ± 0.72 and 9.25 ± 0.44 x 10^3 / µl respectively). A significant difference ($P<0.05$) was found between groups (1, 2) and groups (3 and the control group) table 4-8.

In differential count lymphocyte showed the lower count in lambs born to ewes in groups (1 and 2) with means that reached (48.40 ± 4.35 and 51.80 ± 2.92 % respectively) in comparison to lambs born to ewes of groups (3 and the control group) in which the means were (83.80 ± 1.98 and 68.94 ± 1.05 % respectively). A significant difference ($P<0.05$) was found between groups (1, 2) and groups (3 and the control group). A significant difference ($P<0.05$) was also found between group (3) and the control group table 4-8.

Neutrophil showed the higher count in lambs born to ewes of groups (1 and 2) with means that reached (45.00 ± 5.93 and 43.00 ± 4.06 % respectively) in comparison to lambs born to ewes of groups (3 and the control group) in which the means were (15.70 ± 1.98 and 31.98 ± 1.10 % respectively).

A significant difference ($P<0.05$) was found between groups (1, 2) and groups (3 and the control group). A significant difference ($P<0.05$) was also found between group (3) and the control group table 4-8.

Monocyte showed the higher count in lambs born to ewes of groups (1 and 2) with means that reached (2.20 ± 0.20 and 2.00 ± 0.31 % respectively) in comparison to lambs born to ewes in groups (3 and the control group) in which the means were (0.50 ± 0.00 and 0.50 ± 0.00 % respectively).

A significant difference (P<0.05) was found between groups (1, 2) and groups (3 and the control group) table 4-8.

Eosinophil showed the higher count in lambs born to ewes of groups (1 and 2) with means that reached (4.00 ± 1.94 and 2.80 ± 1.24 % respectively) in comparison to lambs born to ewes of groups (3 and the control group) in which the means were (0.00 ± 0.00 and 0.00 ±0.00 % respectively).

A significant difference (P<0.05) was found between group (1) and groups (3 and the control group) while group (2) showed no significant difference (P<0.05) as compared to other groups table 4-8.

Basophil showed the higher count in lambs born to ewes of groups (1 and 2) with means that reached (0.40 ± 0.24 and 0.40 ± 0.24 % respectively) in comparison to lambs born to ewes of groups (3 and the control group) in which the means were (0.00 ± 0.00 and 0.00 ±0.00 % respectively). No significant difference (P<0.05) was found between different groups table 4-8.

Ewes in groups (1 and 2) showed the lower white blood cell count with means that reached (6.87 ± 0.64 and 9.37 ± 0.68 x 10^3 / µl respectively) as compared with the means of ewes of groups (3 and the control group) which reached (13.40 ± 1.43 and 10.53± 0.12 x 10^3 / µl respectively).

Group (1) showed a significant difference (P<0.05) in comparison to group (3 and the control group). Group (2) also showed a significant difference (P<0.05) in comparison to group (3). No significant difference (P<0.05) was found between group (1) and group (2) and the control group table 4-9.

In differential count lymphocyte showed the lower count in ewes of group (1) in which the mean reached (36.42 ± 4.36 %) in comparison to ewes of groups (2 , 3 and the control group) in which the means reached (74.71 ± 2.46 , 69.64 ± 2.47 and 64.61 ± 1.29 % respectively). Group (1) showed a significant

difference (P<0.05) in comparison to groups (2 , 3 and the control group). Group (2) showed a significant difference (P<0.05) in comparison to the control group. Group (3) showed no significant difference (P<0.05) in comparison to the control group table 4-9.

Neutrophil showed the higher count in ewes of group (1) in which the mean reached (57.42 ± 4.66 %) in comparison to ewes of groups (2, 3 and the control group) in which the means reached (18.71 ± 1.64 , 29.85 ± 2.47 and 34.88 ± 1.29 % respectively). Group (1) showed a significant difference (P<0.05) in comparison to groups (2 , 3 and the control group). Group (2) showed a significant difference (P<0.05) in comparison to groups (3 and the control group). No significant difference (P<0.05) was found between groups (3 and the control group) table 4-9.

Monocyte showed the higher count in ewes of group (2) in which the mean reached (7.28 ± 2.36 %) in comparison to ewes of groups (1 , 3 and the control group) in which the means reached (5.28 ± 1.24 , 0.50 ± 0.00 and 0.50 ± 0.00 % respectively). A significant difference (P<0.05) was found between groups (1 , 2) and groups (3 and the control group) table 4-9.

Eosinophil showed the higher count in ewes of groups (1 and 2) in which the means reached (0.71 ± 0.28 and 0.71 ± 0.28 % respectively) as compared with ewes of groups (3 and the control group) in which the means reached (0.00 ± 0.00 and 0.00 ± 0.00 % respectively). A significant difference (P<0.05) was found between groups (1, 2) and group (3) and the control group table 4-9.

Basophil in ewes of all groups showed the same count with means of (0.00 ± 0.00 %). No significant difference (P<0.05) was found between different groups table 4-9. Blood samples were collected from ewes and their lambs at the time of occurrence of clinical signs of the deficiency in lambs.

The results of total leukocyte count in this study are consistent with results of Mohri *et al*., (2005) who reported that calves injected with vitamin E and selenium at a dose of 300 U vitamin E (α-tocopherol acetate) plus 6 mg selenium (sodium selenite) per 45 kg body weight at 24–48 h and 14 days after birth showed the higher levels of white blood cells at the third and fourth weeks of life compared with the control group (not supplemented with vitamin E and selenium) and this rise in white blood cells could be related to the protection of cell membrane and intracellular organelles by the antioxidant effects of vitamin E and selenium and thus increase the life span of leukocytes.

The results of this study are also in line with results of Emmanuel, (2010) who mentioned that higher total leukocyte counts were observed in animals infected with *haemonchus contortus* and supplemented with selenium , where the neutrophils were responsible for the increase in the total leukocyte count.

In contrast to the result of this study the total white blood cell count significantly increased in lambs which become vitamin E deficient by giving 3 ml cod liver oil daily for 62 to 74 days when vitamin E deficiency was complicated with pneumonia (Culik *et al*., 1951).

The results of differential leukocyte count in this study are agreed with Nemec *et al*., (1990) who reported that lymphocytes are considered the population of the cells most sensitive cells to free radicals due to the high level of free fatty acids in their membranes. In contrast with the results of this study Boa-Amponsem *et al*., (2000) reported that Heterophil:lymphocyte ratios for cockerels fed the higher dietary vitamin E (300 mg/kg) was higher than for those fed the lower dietary vitamin E (10 mg/kg).

Neutrophil, monocyte, eosinophil and basophil showed the higher counts in deficient animals compared to animals in healthy groups these results are in

agreement with Hong and Chow, (1988) who reported that vitamin E and selenium deficient rat developed a marked eosinophilic enteritis and eosinophilia which were not observed in rats fed the basal diet supplemented with either vitamin E (100 or 200 ppm) or selenium (0.1 or 1.0 ppm).

The results of this study are also in line with Whitmore, (1965) who stated that vitamin E deficiency in association with diets of high rancidity produced a pronounced granulocyte response in chinook salmon complete recovery resulted when vitamin E was added to the diet.

The increased count of the granulocytes may be a response to an anemic condition that may have occurred in vitamin E and selenium deficient animals. Dinning and Day, (1957) reported that vitamin E-deficient monkeys exhibited anemia and granulocytosis and in most cases a lymphopenia.

Table (4-8) The total and differential white blood cells counts in newborn lambs.

Groups	mean ±S.E					
	WBCs N x 10^3/µL	**Lymphocyte %**	**Nutrophil %**	**Monocyte %**	**Eosinophil %**	**Basophil %**
Group 1* Born to deficient and vaccinated ewes	2.88 ± 0.12 A	48.40 ± 4.35 A	45.00 ± 5.93 A	2.20 ± 0.20 A	4.00 ± 1.94 A	0.40 ± 0.24 A
Group 2* Born to deficient ewes	2.88 ± 0.18 A	51.80 ± 2.92 A	43.00 ± 4.06 A	2.00 ± 0.31 A	2.80 ± 1.24 AB	0.40 ± 0.24 A
Group 3 Born to vaccinated ewes	9.07 ± 0.72 B	83.80 ± 1.98 B	15.70 ± 1.98 B	0.50 ± 0.00 B	0.00 ± 0.00 B	0.00 ± 0.00 A
Control group Born to control ewes	9.25 ± 0.44 B	68.94 ± 1.05 C	31.98 ± 1.10 C	0.50 ± 0.00 B	0.00 ± 0.00 B	0.00 ± 0.00 A

n=7

*Two lambs died

Different letters mean significant ($P<0.05$) results between different groups

Table (4-9) The total and differential white blood cells counts in ewes.

Groups	mean ±S.E					
	WBCs N x 10^3/µL	**Lymphocyte %**	**Nutrophil%**	**Monocyte %**	**Eosinophil%**	**Basophil%**
Group 1 Deficient and vaccinated ewes	6.87 ± 0.64 A	36.42 ± 4.36 A	57.42 ± 4.66 A	5.28 ± 1.24 A	0.71 ± 0.28 A	0.00 ± 0.00 A
Group 2 Deficient ewes	9.37 ± 0.68 AC	74.71 ± 2.46 B	18.71 ± 1.64 B	7.28 ± 2.36 A	0.71 ± 0.28 A	0.00 ± 0.00 A
Group 3 Vaccinated ewes	13.40 ± 1.43 B	69.64 ± 2.47 BC	29.85 ± 2.47 C	0.50 ± 0.00 B	0.00 ± 0.00 B	0.00 ± 0.00 A
Control group	10.53 ± 0.12 C	64.61 ± 1.29 C	34.88 ± 1.29 C	0.50 ± 0.00 B	0.00 ± 0.00 B	0.00 ± 0.00 A

n=7
Different letters mean significant (P<0.05) results between different groups

4-5-Creatine Kinase and Aspartate Aminotransferse:

The results of this study revealed an increase in serum activity of creatine kinase in lambs born to ewes of groups (1 and 2) with means that reached (2756.52 ± 20.79 and 2920.80 ± 17.45 U/L respectively) as compared with those from lambs of groups (3 and the control group) which were (206.40 ± 1.42 and 292.52 ± 1.20 U/L respectively) these results showed significant differences between groups (1 and 2) and between groups (3 and the control group).The results showed a significant difference (P<0.05) between different four groups table 4-10.

The results also revealed that there was an increase in serum activity of aspartate aminotransferase in lambs of groups (1 and 2) with means that reached (145.40 ± 7.94 and 144.80 ± 5.28 U/L respectively) as compared with those from lambs of groups (3 and the control group) which were (73.85 ± 4.23 and 72.85 ± 2.33 U/L respectively) these results showed significant differences between groups (1, 2) in comparison with groups (3 and the control group) ($P<0.05$) table 4-10.

The results of creatine kinase revealed an increase in serum activity of this enzyme in ewes of groups (1 and 2) with means that reached (2070.51 ± 22.22 and 2776.52 ± 30.66 U/L respectively) as compared with those from ewes of groups (3 and the control group) which were (209.56 ± 2.24 and 211.07 ± 2.23 U/L respectively) these results showed significant differences among groups (1 and 2) and in comparison with groups (3 and the control group) ($P<0.05$) table 4-11.

The results of aspartate aminotransferase revealed an increase in serum activity of this enzyme in ewes of groups (1 and 2) with means that reached (143.71 ± 4.28 and 133.85 ± 3.83 U/L respectively) as compared with those from ewes of groups (3 and the control group) which were (74.42 ± 2.27 and 69.14 ± 2.78 U/L respectively) these results showed significant differences between groups (1 , 2) and groups (3 and the control group) ($P<0.05$) table 4-11.

The results of this study showed that there was an increase in serum activities of creatine kinase and aspartate aminotransferase in selenium and vitamin E deficient ewes and their newborn lambs, this is in agreement with Wright and Bell, (1964) and Buchanan-Smith *et al*., (1969) they reported that aspartate aminotransferase activity in plasma increased in vitamin E-deficient

ewe lambs, the elevated plasma activities of aspartate aminotransferase and creatine kinase together confirmed the presence of necrosis in skeletal muscle.

The results in this study are in concert with Kuttler *et al*., (1961) and Cardinet, (1971) they mentioned that increased serum creatine kinase activity was recorded in lambs affected by white muscle disease. This enzyme is involved in helps providing energy for muscle contraction. The duration of elevation following muscle injury is considerably less than for aspartate aminotransferase and the increased activity of aspartate aminotransferase, which appear to be proportional to the degree of muscle fiber degeneration , was used in the diagnosis of nutritional muscular dystrophy in ruminants.

Table (4-10) Creatine kinase and aspartate aminotransferase activities in newborn lambs.

Groups	mean ±S.E	
	CK (U/L)	AST (U/L)
Group 1* Born to deficient and vaccinated ewes	2756.52 ± 20.79 A	145.40 ± 7.94 A
Group 2* Born to deficient ewes	2920.80 ± 17.45 B	144.80 ± 5.28 A
Group 3 Born to vaccinated ewes	206.40 ± 1.42 C	73.85 ± 4.23 B
Control group Born to control ewes	292.52 ± 1.20 D	72.85 ± 2.33 B

n=7

*Two lambs died

Different letters mean significant (P<0.05) results between different groups

Table (4-11) Creatine kinase and aspartate aminotransferase activities in ewes

Groups	mean ±S.E	
	CK (U/L)	AST (U/L)
Group 1 Deficient and vaccinated ewes	2070.51 ± 22.22 A	143.71± 4.28 A
Group 2 Deficient ewes	2776.52 ± 30.66 B	133.85 ± 3.83 A
Group 3 Vaccinated ewes	209.56 ± 2.24 C	74.42 ± 2.27 B
Control group	211.07 ± 2.23 C	69.14 ± 2.78 B

n=7

Different letters mean significant (P<0.05) results between different groups

4-6-Milk somatic cell count (SCC):

Milk samples of ewes of groups (1 and 2) showed a higher somatic cell count (1.80 ± 0.16 and 2.09 ± 0.23 $x10^6$/ml respectively) than that of ewes of groups (3 and the control group) in which the means reached (0.69 ± 0.00 and 0.62 ± 0.01 $x10^6$/ml respectively).

The results showed a significant difference (P<0.05) between groups (1 and 2) in comparison with groups (3 and the control group) table 4-12.

The results of this study agreed with Smith *et al*., (1985) who mentioned that selenium and vitamin E supplemented heifers had significantly lower milk somatic cell counts compared with unsupplemented heifers.

The high somatic cell count in this study reflected the lack of the protective role of vitamin E and selenium in the deficient animals. Craven and Williams, (1985) stated that when bacteria gain entrance to the udder, an influx of white blood cells occurs to fight off the infection. Peroxides (and other substances) are produced by the white blood cells to help destroy the pathogens.

If these peroxides are not controlled, this can be dangerous to healthy cells and tissue. Vitamin E and selenium are necessary to help the white blood cells reduce the peroxides to safe substances and to continue destruction of invading pathogens. When an animal is deficient in vitamin E and/or selenium, this function of white blood cells is impaired.

Table (4-12) Somatic cell count in ewes' milk

Groups	mean ±S.E Somatic cell count $x10^6$/ml
Group 1 Deficient and vaccinated ewes	1.80 ± 0.16 A
Group 2 Deficient ewes	2.09 ± 0.23 A
Group 3 Vaccinated ewes	0.69 ± 0.00 B
Control group	0.62 ± 0.01 B

n=7

Different letters mean significant (P<0.05) results between different groups

Table 4-13 Estrus synchronization results

Animals groups	Number of ewes	% estrus synchronization	Number of ewes mated	Number of ewes lambed
Group 1 Deficient and vaccinated ewes	10	100	10	9
Group 2 Deficient ewes	10	100	10	7
Group 3 Vaccinated ewes	10	100	10	8
Control group	10	100	10	7

Conclusions

1. Feeding cod liver oil mixed with ground corn for two months during pregnancy and one month after parturition was an effective method of producing an experimental vitamin E and selenium deficiency in Awassi ewes and their newborn lambs.
2. Sudden death together with low serum levels of selenium and vitamin E in lambs born to vitamin E and selenium deficient ewes were characteristic features of vitamin E and selenium deficiency.
3. Vitamin E and selenium deficiency affects udder health and decreases wool, meat and milk production of the deficient ewes.
4. Maternal status of vitamin E and selenium had an apparent effect on vitamin E and selenium status of newborn lambs, vitamin E and selenium are essential antioxidants and their deficiency exposes sheep industry to serious losses.
5. Subclinical vitamin E and selenium deficiency in ewes can be diagnosed depending on the presence of low serum levels of vitamin E and selenium, and elevated serum level of muscle enzymes (CK and AST) before appearance of clinical signs of deficiency.
6. Vitamin E and selenium deficiency had an apparent negative effect on immunity represented by lower phagocytic activity of polymorphonuclear leukocytes in the deficient ewes and lambs than that in healthy ewes and lambs.

Recommendations

1. Carrying out a histopathological study of tissues and organs of vitamin E and selenium deficient animals.
2. Involving of Electrocardiography (ECG) as a diagnostic tool.
3. Estimation of serum enzymes other than Creatine Kinase and Aspartate Aminotransferase such as Lactate Dehydrgenase, Alkaline Phosphatase and minerals other than selenium such as calcium as they could also be considered as an indicator for vitamin E and selenium deficiency.
4. Direct determination of free radicals in the biological tissues of the vitamin E and selenium deficient animals.
5. Study further immunological parameters such as the estimation of interleukins in blood of vitamin E and selenium deficient animals.
6. More sophisticated studies are recommended to be done such as the effect of vitamin E and selenium deficiency on gene transcription in T-Lymphocyte.

References

Aitken, I. D. (2007). Selenium and vitamin E deficiency, incidence/natural occurrence. In: Diseases of Sheep. Blackwell publishing, Fourth Edition. Pp: 387.

Allen, W. H.; Bradley, R. and Berrett, S. (1975). Degenerative myopathy with myoglobinutia in yearling cattle. Br. Vet. J. 131(3): 292.

Allison, R.D. and Laven, R.A. (2000). Effect of vitamin E supplementation on the health and fertility of dairy cows: a review. Vet. Rec. 147(25): 703–708.

Ammerman, C. B. and Miller, S. M. (1975). Selenium in ruminant nutrition. A review. J. Dairy Sci. 58 (10): 1561-1577.

Anderson, P. H.; Bradley, R.; Berret, S. and Paterson., D. S. P. (1977). The sequence of myodegeneration in nutritional myopathy in the older calf. Br. Vet. J. 133 (2): 160-165.

Arechiga, C. G.; Ealy, A. D. and Hansen, P. J. (1994). Efficacy of vitamin E and glutathione for thermoprotection of murine morulae. Theriogenology. 41: 1545–1553.

Arova, K. B.; Gresakova, L.; Falx, S.; Levkut, M. and Leng, L. (2003). Urinary selenium excretion in seleniteloaded sheep and subsequent Se dynamics in blood constituents. Reprod. Nutr. Dev. 43 : 385-393.

Azizi, E. S.; Klesius, P. H. and Frandsen, J. C. (1984). Effects of selenium on polymorphonuclear leukocyte function in goats. Am. J. Vet. Res. 45 (9): 1715-1719.

Baumgartner, W.A. (1979). Antioxidants, cancer and the immune response. In: Trace Metals in Health and Disease (ed. N. Kharasch), Pp: 295. Raven Press, New York.

Bendich, A. (1990). Antioxidants vitamins and their functions in immune response. Adv. Exp. Med. Biol. 262: 35-55.

Beytut, E.; Karataş, F. and Beytut, E. (2002). Lambs with white muscle disease and selenium content of soil and meadow hay in the region of Kars. Turkey Vet. J. 163(2): 214-217.

Birringer, M.; Pilawa, S. and Flohe, L. (2002). Trends in selenium biochemistry. Natural Products Reports. 19 : 693–718.

Blincoe, C. and Dye, W. B. (1958). Serum transaminase in white muscle disease. J. Anim. Sci. 17(1): 224.

Blood, D. C. and Henderson, J. A. (1968).Veterinary Medicine. Third Edition. Pp: 740-746. London: Bailliere, Tindall and Cassell.

Boa-Amponsem, K.; Price, S.E.H.; Picard, M.; Geraert, P. A. and Siegel, P. B.(2000) Vitamin E and immune responses of broiler pureline chickens. Poultry Science. 79:466–470.

Bostedt, H. and Schramel, P. (1990). The importance of selenium in the prenatal and postnatal development of calves and lambs. Biol. Trace Elem. Res. 24 (2): 163-171.

Boyne, R. and. Arthur, J. R. (1979). Alterations of neutrophil function in selenium-deficient cattle. J. Comp. Pathol. 89 : 151-158.

Boyne, R. and Arthur, J. R. (1986). The response of selenium-deficient mice to *Candida albicans* infection. J. Nutr. 116 (5):816-822.

Braun, U.; Forrer, R.; Fürer, W. and Lutz, H. (1991). Selenium and vitamin E in blood sera of cows from farms with increased incidence of disease. Vet. Rec. 128 (23): 543-547.

Brin, M.; Horn, L. R.; and Barker, M.0. (1974). Relationship between fatty acid composition of erythrocytes and susceptibility to vitamin E deficiency. Am. J. Clin. Nutr. 27 (9): 945-951.

Buchanan-Smith, J. G. ; Nelson, E. C.; Osburn, B. I.; Wells, M. E. and Tillman, A. D. (1969). Effects of vitamin E and selenium deficiencies in sheep fed a purified diet during growth and reproduction. J. Anim. Sci. 29 (5): 808-815.

Bunnell, R. H. ; Keating, J. B. and Quaresimo, A. J. (1968). Alpha-tocopherol content of feedstuffs. J. Agric. Fd Chem. 16 (4): 659-664.

Cardinet, G. H. (1971). Skeletal Muscles. In Clinical Biochemistry of Domestic Animals, 2nd Edition. 2 Pp:155-177. Edited by J. J. Kaneko and C. E. Cornelius. New York: Academic Press.

Cardinet, G. H.; Litterell, J. F. and Freedland, R. A. (1967). Comparative investigations of serum creatine phosphokinase and glutamic-oxaloacetic transaminase activities in equine paralytic myoglobinuria. Res. Vet. Sci. 8 : 219-226.

Carr, A. C.; Zhu, B. Z. and Freim, B. (2000). Potential antiatherogenic mechanisms of ascorbate (vitamin C) and α-tocopherol (vitamin E). Circ. Res. 87 (5) : 349-354.

Carter, D. L.; Brown, M. J.; Allaway, W. H. and Caryl, E. E. (1968). Selenium content of forage and hay crops in the Pacific Northwest. Agronomy J. 60 : Pp: 532-534.

Cary, C. A. (1939). The Relation of Vitamin E to Nutritional Paralysis: Rpt. of the Chief of the Bureau of Dairy Industry, U. S. Dept. of Agric., Bureau of Dairy Industry, Div. Nutrition and Physiology. Pp:24.

Chan, A. C.; Chow, C. K. and Chiu, D. (1999). Interaction of antioxidant and their implication in genetic anemia. Proc. Soc. Exp. Biol. Med. 222 (3): 274–82.

Chariot, P. and Bignani, O. (2003). Skeletal muscle disorders associated with selenium deficiency in humans. Muscle & Nerve. 27 (6) : 662-668.

Chew, B. P. (1995). Antioxidant vitamins affect food animal immunity and health. J. Nutr. 125 : 1804-1808.

Chew, B. P. (1996). Importance of antioxidant vitamins in immunity and health in animals. Anim. Feed Sci. Tech. 59 : 103-114.

Chow, C K.; Reddy, K. and Tappel, A. L. (1969). Effect of dietary vitamin E on the activity of glutathione peroxidase in vitro and in vivo studies. J. Clin. Invest. 48 : 1957.

Coburn Williams, M. and Mayland, H. F. (1992). Selenium absorption by two-grooved milkvetch and Western wheatgrass from selenomethionine, selenocystine, and selenite. J. Range Manage. 45 : 374-378.

Coles, E. H. (1986). Veterinary Clinical Pathology.4th edition, W. B. Saunders Company, Philadelphia. Pp: 436-437.

Craven, N., and Williams, M. R. (1985). Defenses of the bovine mammary gland against infection and prospects for their enhancements. Vet. Immunol. Immunopath. 2 : 71.

Culik, R. F.; Bacigalupo, A.; Thorp, F.; J., R., Luecke, W. and Nelson, R. H. (1951). Vitamin E deficiency in the lamb. J. Anim. Sci. 10 : 1006-1016.

Deger, Y.; Mert, H.; Mert, N.; Yur, F.; Kozat, S.; Yoruk, I. and Sel, T. (2008). Serum selenium, vitamin E, and sialic acids concentrations in lambs with white muscle disease. Biol. Trace Elem. Res., 121: 39-43.

Demirel, G; Wachira, A.M.; Sinclair, L.A.; Wilkinson, R.G.; Wood, J.D. and Enser, M. (2004) Effects of dietary n-3 polyunsaturated fatty acids, breed and dietary vitamin E on the fatty acids of lamb muscle, liver and adipose tissue. Br. J. Nutr. 91: 551–565.

Dinning, J. S. and Day, P.L. (1957) Vitamin E deficiency in the monkey I. Muscular Dystrophy, Hematologic Changes and the excretion of Urinary Nitrogenous Constituents. J. Exp. Med. Pp:395-402.

El-Gaafrawy, A. M.; Nagwa, A.; El-Banna, M. K. and Ibrahim, I. L. (2000). Effects of selenium and vitamin E supplementation on immune response and performance of Baladi calves. Proc. Conf. Anim. Prod. Pp : 271-276.

Emmanuel, V. DE C. (2010). Neutrophils activity and oxidative stress of experimentally infected lambs by *Haemonchus contortus* and supplemented with selenium and vitamin E, Master's Dissertation Santa Maria, Fevereiro.

Enjalbert, F. ; Lebreton, P. ; Salat, O. ; Meschy, F. and Schelcher, F. (1999). Effect of pre- or postpartum selenium supplementation on selenium status in beef cows and their calves. J. Anim. Sci. 77 : 223–229.

Erskine, R. J.; Eberhart, R. J.; Grasso, P. J. and Scholz, R. W. (1989). Induction of Escherichia coli mastitis in cows fed selenium-deficient or selenium-supplemented diets. Am. J. Vet. Res. 50 : 2093.

Erwin, E. S.; Sterner, W. R.; Gordon, S. L.; Machlin, J. and Tureen, L. L. (1961). Etiology of Muscular Dystrophy in the Lamb and Chick. J. Nutr. 75 : 61.

Ewan, R. C.; Baumann, C. A. and Pope. A. L. (1968). Effects of selenium and vitamin E on nutritional muscular dystrophy in lambs. J. Anim. Sci. 27 : 751.

Ferencik, M. and Ebringer, L. (2003). Modulatory effects of selenium and zinc on the immune system. - Folia Microbiol.(Praha). 48 (3):417-426.

Fischer, W. C. and Whanger, P. D. (1977). Effects of selenium deficiency on vitamin E metabolism in rats. J. Nutr. Sci. Vitaminol. 23 (4): 273-280.

Forbes, R. M. and Draper, H. H. (1958). Production and study of vitamin E deficiency in the baby pig. J. Nutr. 65 : 535-545.

Gallo-Torres, H. (1972). Vitamin E in animal nutrition. Int. J. Nutr. Res. 42: 312-323.

Gardner, R. W., and Hogue, D. E. (1967). Milk levels of selenium and vitamin E related to nutritional muscular dystrophy in the suckling lamb. J. Nutr. 93 : 67.

Giovannucci, E. (1998). Selenium and risk of prostate cancer. The Lancet. 352 : 755-756.

Gropper, S. S.; Smith, J. and Groff J. (2005). Advanced Nutrition and Human Metabolism: Glutathione peroxidase.4thed.Wadsworth. Belmont, CA. 458 USA.

Gurr, M. I. and Harved, J. L. (1991). Lipid Biochemistry:An Introduction. Chapman & Hall. New York, Pp: 186-187.

Gyang, E. O.; Stevens, J. B.; Olson, W. G.; Tsitsamis, S. D. and Usenik, E. A. (1984). Effects of selenium-vitamin E injection on bovine polymorphonucleated leukocytes phagocytosis and killing of *Staphylococcus aureus*. Amer. J. Vet. Res. 45 : 175-177.

Hadden, J. W. (1987). Neuroendocrine modulation of the thymus dependant immune system. Ann. NY. Acad. Sci. 496.

Hadlow, J.W. (1974). Myopathies of animals - a brief view. In: Homburger, F., Sucas, I., Karger, S. (Eds.), Comparative Pathology of the Heart, Advanced Cardiology. 13 Basel, Pp: 68-80.

Hamliri, A.; Olson, W. G.; Johnson, D. W. and Kessabi, M. (1990). Evaluation of biochemical evidence of congenital nutritional myopathy in two-week prepartum foetuses from selenium-deficient ewes. Am. J. Vet. Res. 51: 1112-1115.

Heikkila, R. E.; Mezick, J. A. and Cornwell, D. G. (1971). Destruction of specific membrane phospholipids during peroxidative hemolysis of vitamin E deficient erythrocytes. Physiol. Chem. Phys. 3 : 93-97.

Herdt, T. H. ; Rumbeiha, W. and Braselton, W. E. (2000). The use of blood analyses to evaluate mineral status in livestock. Vet. Clin. North Am. Food Anim. Pract. 16 (3): 423-44.

Hidiroglou, M. (1977). Effect of diet on the metabolism of labelled tocopherol in sheep. Br. J. Nutr. 37: 215- 225.

Hidiroglou, M. ; Jenkins, K. J. and Corner, A. H. (1972). Control of nutritional muscular dystrophy in lambs by vitamin E implantations. Can. J. Anim. Sci. 52 : 511.

Hidiroglou, M. ; Jenkins, K. J. and Lessard, J. R. (1970). Metabolism of vitamin E in sheep. Br. J. Nutr. 24 : 917.

Hidiroglou, M.; Nelson, F. C.; Lessard, J. R. and Taylor, P. A. (1967). Serum Transaminase Activity in Calves Affected by Nutritional Muscular Dystrophy in Northern Ontario. Can. Vet. J. (8) 3 : 62-69.

Hidiroglou, N.; Cave, N. and Atwal, A.S. (1992). Comparative vitamin E requirements and metabolism in livestock. Annales de Recherche Veterinaire (Abstract). 23: 337–42.

Hong, C.B. and Chow, C.K. (1988) Induction of eosinophilic enteritis and eosinophilia in rats by vitamin E and selenium deficiency. Exp. Mol. Pathol. 48 (2):182-192.

Hopkins, L. L.; J., R.; Pope, A. L. and Baumann, C. A. (1964). Contrasting Nutritional Responses to Vitamin E and Selenium in Lambs. J. Anim. Sci. 23 : 674-681.

Horn, L. R.; Barker, M. O.; Reed, G. and Brin, M. (1974). Studies on peroxidative hemolysis and erythrocyte fatty acids in the rabbit: effect of dietary PUFA and vitamin E. J. Nutr. 104 : 192-201.

Horvath, P. M. and C, I. P. (1983). Synergistic effect of vitamin E and selenium on the chemo prevention of mammary carcinogenesis in rats. Cancer Res. 43 (11): 5335-5341.

Hoshino,Y.; Ichijo, S.; Osame, S. and Takahashi, E. (1989). Studies on serum Tocopherol, Selenium levels and blood Glutathione Peroxidase Activities in calves with white muscle disease. Jpn. J. Vet. Sci. 51(4):741-748.

Huang, C. J.; Cheung, N. S. and Lu, V. R. (1988). Effects of deteriorated frying oil and dietary protein levels on liver microsomal enzymes in rats. J. Am. Oil Chem. Soc. 65 : 1796-1803.

Jacobs, L. W. (1989). Selenium in Agriculture and the Environment, Soil Science Society of America Special Publication 23, SSSA , Madison ,WI.

Jaja, S. I.; Aigbe, P. E.; Gbenebipse, S. and Temiyp, E. O. (2005). Changes in erythrocytes following supplementation with alpha-tocopherol in children suffering from sickle cell anemia. Niger. Postgrad. Med. J. 12 (2) : 110–4.

Jordan, R. M. (1970). Selenium-vitamin E interrelationships in preventing muscular dystrophy in lambs and calves. Proc. of the thirty-first annual Minnesota Nutrition Conference, Minneapolis. Pp: 29-42.

Judith, L. C.; Robert, G. W.; Eleni Kasapidou; Sandra, E. P.; Alexander, M. M. and Liam, A. S. (2005) The effect of dietary vitamin E and fatty acid supplementation of pregnant and lactating ewes on placental and mammary transfer of vitamin E to the lamb. Br. J. Nutr. 93: 549–557.

Kamada, H. ; Nishimura, K. ; Krongyuti, P. ; Sukkasame, P. ; Phoengpong, N. and Intramanee, S. (2000). Selenium status of soil, herbage and beef cattle in southern Thailand. Asian-Austr.J. Anim. Sci. 13 : 757-760.

Kuricova, S.; Boldiiarova, K.; Gresakova, L.; Bobaek, R.; Levkut, M. and Leng, L. (2003). Chicken selenium status when fed a diet supplemented with Se-yeast. Acta. Vet. Brno. 72 : 339-346.

Kuttler, K. L.; Marble, D. W. and BIncoe, C. (1961). Serum and tissue residues following selenium injections in sheep. Am. J. vet. Res. 22 : 422.

Leng, L.; Bobcek, R.; Kuricova, S.; Boldiiarova, K.; Gresakova, L.; Sevaikova, Z.; Revajova, V.; Levkutova, M. and Levkut, M. (2003). Comparative metabolic and immune responses of chickens fed diets containing inorganic selenium and Sel-PlexTM organic selenium. In: Nutritional Biotechnology in the Feed and Food Industries. Proceedings of Alltech`s Nineteenth Annual Symposium, Nottingham University Press, Notingham, UK, 131-145.

Maas, J. P. (1983). Diagnosis and management of selenium responsive diseases in cattle. Compen. Contin. Educ. Pract. Vet. 5 : 383.

Maas, J., and Valbery, S. I. (2009).Nutritional Myodegeneration in Large Animals Internal Medicine,4thed (ed. Smith, B.P) Mosby, Elsevier. Pp: 1405-1408.

MacPherson, A. (1994). Selenium, vitamin E and biological oxidation. In: (Ed. P. C. Garnsworthy and D. J. A. Cole) Recent Advances in Nutrition. Nottingham University Press, Nottingham. Pp: 3-30.

Malbe, M.; Klaassen, M.; Fang, W.; Myllys, V.; Vikerpuur, M.; Nyholm, K.; Sankari, W.; Suoranta, K. and Sandholm, M. (1995). Comparisons of selenite and selenium yeast feed supplements on Se-incorporation, mastitis, and leukocyte function in Se-deficient dairy cows. J. Vet. Med. (Ser. A) 42 : 111-121.

Mason, K. E. and Bryan, W. L. (1940). Placental and mammary transfer of vitamin E in the rat. J. Nutr. 20 : 501.

Max, J. P.; Douglas D. B.; Xin, Z. and Jai-Wei L. (2003). The bovine neutrophil: Structure and function in blood and milk. Vet. Res. 34: 597–627.

Mayland, H. F. (1994). Selenium in plant and animal nutrition, In: Selenium in the environment. Frankenberger JR., W. T. and Benson, S. (eds,). Marcell Decker Inc., New York. Pp: 29-45.

McDowell, L. R, (1989). Vitamin E. In: Vitamins in Animal Nutrition, Academic Press, Inc, San Diego.

McDowell, L. R. (1992). Minerals in Animal and Human Nutrition. Academic Press Inc. Harcourt Brace Jovanovich Publishers, San Diego, CA, USA.

McDowell, L. R. (2000). Vitamin E. In vitamins in animal and human nutrition, McDowell L.R. (ed.), 2nd edition, Iowa State University Press/Ames, USA. Pp : 155-225.

McDowell, L. R. (2003). Minerals in Animal and Human Nutrition. 2nd edition. Elsevier Science B.V., Amsterdam, The Netherlands.

McDowell, L. R.; Williams, S.N.; Hidiroğlu, N.; Njeru, C. A.; Hill, G. M.; Ochoa, L. and Wilkinson, N.S. (1996). Vitamin E supplementation for the ruminant. Anim. Feed Sci. Tech. 60 : 273-296.

McKenzie, R. C. ; Rafferty, T. S. and Beckett, G. J. (1998). Selenium: an essential element for immune function. Trends Immunology Today. 19 (8) : 342.

McMurray, C. H. and McEldowney, P.K. (1977). A possible prophylaxis and model for nutritional degenerative myopathy in young cattle. Br. Vet. J. 133 (5): 535-542.

McMurray, C. H.; Rice, D. A. and Blanchflower, W. J. (1980). Changes in plasma levels of linoleic acids in calves recently introduced to spring pasture. Proc. Nutr. Soc. 39 : 65A (Abstr).

Melville, R. S. and Hummel, J. P. (1951). Creatine and glycocyamine metabolism in rabbits in vitamin E deficiency. J. Biol. Chem. 191 : 383.

Menzies, P.; Langs, L.; Boermans, H.; Martin, J. and McNally, J. (2004). Myopathy and hepatic lipidosis in weaned lambs due to vitamin E deficiency. Can. Vet. J. 45:244–247.

Metcalf ,J.A. ;Gallin ,J.I.;Nauseef,.M.and Root ,R.K.(1986) .Laboratory Manual of Neutrophils Function .Raven Press .Newyork,USA .,pp:191.

Meydani, M. (1995). Vitamin E. The Lancet. 345: Pp: 170–175.

Meydani, S. N. and Blumberg, J. B. (1993). Vitamin E and the immune response. In: Cunningham-Rundles S. ed. Nutrient modulation of the immune response. New York: Marcel Dekker. 223-38.

Meydani, S. N., Han, S. N. and Wu, D. (2005). Vitamin E and immune response in the aged: molecular mechanism and clinical implications. Immunol. Rev. 205 : 269–284.

Meyer, D. J. and Harvey, J. W. (1998). Veterinary laboratory medicine: interpretation and diagnosis. Second edition, W. B. Saunders Company. Pp: 373.

Micke, O. ; Buntzel, J. ; Bruns, F. ; Glatze, M. ; Schonekaes, K. G.; Kisters, K. and Mucke, R. (2003). Selenium in oncology – past, presence and future. 4th International Symposiumon Trace Elements in Human: New Perspectives, Athenes, Greece. Proceedings Book (Eds. S. Ermidou-Pollet, S. Pollet), Part I. Pp: 259-266.

Milad, K. ;Racz, O;Sipulova,V;Bajova,V.and Kovac, G. (2001) Effect of vitamin E and selenium on blood glutathione peroxidase and some immunological parameters in sheep. Vet. Med.-Czech. 46 (1):1-5.

Miller, J. K.; Ramsey, N. and Madsen, F. C. (1988). The trace elements In: the Ruminant Animal: Digestive Physiology and Nutrition, D, C, Church (ed,), Englewood Cliffs, NJ. Prentice-Hall Inc. Pp: 342.

Mohri, M.;Seifi, H. A. and Khodadadi, J. (2005) Effects of preweaning parenteral supplementation of vitamin E and selenium on hematology, serum proteins, and weight gain in dairy calves. Comp. Clin. Pathol. 14: 149–154.

Molnar, J.; Macpherson, A. and Molnar, P. (1998). The effects of selenium supplementation in feeding of lambs. Acta. Alimentaria. 24: 167-79.

Morris, J. G.; Cripe, W. S.; Chapman, H. L.; Walker, D. F.; Armstrong, J. B.; Alexander, J. D.; Miranda, R.; Sanchez, A.; Sanchez, B.; Blair-West, J. R. and Denton. D. A. (1984). Selenium deficiency in cattle associated with Heinz bodies and anemia. Science. 223 : 491-493.

Murray, R.K.; Granner, D.K.; Mayes, P.A. and Rodwell, V.W. (2003). Vitamin E Is the Major Lipid-Soluble Antioxidant in Cell Membranes & Plasma Lipoproteins. In: Harper's Illustrated Biochemistry, twenty-sixth edition. Lange Medical Books/McGraw-Hill Medical Publishing Division. New York Chicago San Francisco Lisbon London Madrid Mexico City Milan New Delhi San Juan Seoul Singapore Sydney Toronto. Pp : 486.a

Murray, R.K.; Granner, D.K.; Mayes, P.A. and Rodwell, V.W. (2003). Vitamin E does not have a precisely defined metabolic function. In: Harper's Illustrated Biochemistry, twenty-sixth edition. Lange Medical Books/McGraw-Hill Medical Publishing Division. New York Chicago San Francisco Lisbon London Madrid Mexico City Milan New Delhi San Juan Seoul Singapore Sydney Toronto. Pp : 495.b

National Research Council (N.R.C) (1971). Selenium in Nutrition. National Academy of Sciences - National Research Council, Washington, DC.

National Research Council (N.R.C) (2001). Selenium in Nutrition. National Academy of Sciences - National Research Council, Washington, DC.

Neal, R. H. (1995).Selenium. In Heavy Metals in Soils (B. J. Alloway, Ed.) Blackie Academic and Proffessional, London, Pp: 260-283.

Nemec, M.; Hidiroglou, M.; Nielsen, K. and Proulx, J. (1990). Effect of vitamin E and selenium supplementation on some immune parameters following vaccination against brucellosis in cattle. J. Anim. Sci. 68 : 4303-4309.

Nockels, C. F. (1989). Stress, disease and vitamin E requirements. Proc. Twentieth Annual Convention of American Assoc. of Bovine Prac. Kansas City, Mo, USA.

Noguchi, T.; Langevin, M. L.; Combs Jr., G. F. and Scott, M. L. (1973). Biochemical and histochemical studies of Se deficient pancreas in chicks. J. Nutr. 103 : 444-453.

Norheim, G. and Haugen, A. (1986). Precise determination of selenium in tissues using automated wet digestion and automated hydride generator-atomic absorption spectroscopy system. Acta. Pharmacology Toxicology. 59 (7): 610 – 612.

Norton, S. A., and McCarthy, F. D. (1986). Use of injectable vitamin E and selenium-vitamin E emulsion in ewes and suckling lambs to prevent nutritional muscular dystrophy. J. Anim. Sci. 62 : 497-508.

Oldfield, J. E. (1999). Selenium World Atlas, Selenium-Tellurium Development Association, Grimbergen, Belgium.

Olson, S. E. and Seidel, G. E. (2000). Culture of in vitro produced bovine embryos with vitamin E improves development in vitro and after transfer to recipients. Biology of Reproduction. 62 : 248–252.

Ono, K. (1985). Effects of large dose vitamin E supplementation on anemia in hemodialysis patients. Nephron. 40 (4) : 440–5.

Osame, S.; Ohtani, T.; Ichijo, S. (1990). Studies on serum tocopherol and selenium levels and blood glutathione peroxidase activities in lambs with white muscle disease. Japanese. J. Vet. Sci. 15: 65–71.

Ostrea, E. M.; Balun, J. E.; Winkler, R. and Porter, T. (1986). Influence of breast feeding on the restoration of the low serum concentration of vitamin E and β-carotene in the newborn infant. Am. J. Obstet. Gynecol. 154 : 1014.

Pamukçu, T.; Sel, T. and Yarım, G. (2001). Blood serum concentration of selenium and glutathione peroxidase activity in Akkaraman sheep. Tr. J. Vet. Anim. Sci. 25 : 731-734.

Panousis, N.; Giadinis, N.; Roubies, N.; Fytianou, A.; Kalaitzakis, E.; Pourliotis, K.; Polizopoulou, Z. and Karatzias, H. (2007) Selenium, vitamin E and vitamin A status in dairy sheep reared under different feeding systems in Greece. J. Vet. Med. 54 (3): 123-127.

Pavlata, L.; Illek, J. and Pechova, A. (2001). Blood and tissue selenium concentrations in calves treated with inorganic or organic selenium compounds - A comparision. Acta. Vet. Brno. 70: 19–26 a.

Pavlata, L.; Pechova, A. and Illek, J. (2001). Muscular dystrophy in dairy cows following a change in housing technology. Acta. Vet. Brno. 70: 269–275 b.

Pavlata, L. ; Illek, J. ; Pechova, A. and Matujiaek, M. (2002). Selenium status of cattle in the Czech Republic. Acta. Vet. Brno. 71 : 3–8.

Pavlata, L.; Prasek, J.; Filipek, J. and Pechova, A. (2004). Influence of parenteral administration of selenium and vitamin E during pregnancy on selected metabolite parameters and colostrum quality in dairy cows at parturition. Veterinarni Medicina. 49:149–155.

Pavlata, L. ; Podhorsky, A. ; Pechova, A. and Chomat, P. (2005). Differences in the occurrence of selenium, copper and zinc deficiencies in dairy cows, calves, heifers and bulls. Vet. Med. Czech. 50 : 390–400.

Pavlata, L.; Prasek, J.; Podhorsky, A.; Pechova, A. and Haloun, T. (2003). Selenium metabolism in cattle: maternal transfer of selenium to newborn calves at different selenium concentrations in dams. Acta. Vet. Brno. 72 : 639-646.

Pechova, A.; Misurova, L.; Pavlata, L. and Dvorak, R. (2008). Monitoring of changes in selenium concentration in goat milk during short-term supplementation of various forms of selenium. Biol. Trace Elem. Res. 121 : 180–191.

Pehrson, B.; Hakkarainen, J. and Blomgren, L. (1990). Vitamin E status in newborn lambs with special reference to the effect of DL-a-tocopheryl acetate supplementation in late gestation. Acta. Vet. Scand. 31 : 359.

Politis, I.; Hidiroglou, M.; Batra, T. R., Gilmore, J. A.; Gorewit, R. C. and Scherf, H. (1995). Effects of vitamin E on immune function of dairy cows. Amer. J. Vet. Res. 56 : 179-184.

Pritchard, E. T. and Singh, H. (1960). Lipid peroxidation in tissues of vitamin E deficient rats. Biochem. Biophys. Res. Commun. 2 : 184-188.

Pruiett, S. P. ; Morrill, J.L.; Blecha, F.; Reddy, P.G.; Higgins, J.; and Anderson, A.V. (1989). Effect of vitamin C and E in milk replacer on lymphocyte and nutrophil function in bull calves. J. Anim. Sci. 67 (Suppl. 1) : 243.

Pugh, D. G. (2002). Sheep and Goat Medicine,1st Ed., Saunders, Philadelphia. Pp: 246-248.

Putnam, M. E. and Comber, N. (1987).Vitamin E .Vet. Rec. 121: 541-545.

Quigley, J. D. and Drewry, J. J. (1998) Nutrient and immunity transfer from cow to calf pre- and postcalving. J. Dairy Sci. 81:2779–2790.

Qureshi, Z.L. ; Lodhi, L.A.; Samad, H.A.; Naz, N.A. and Nawaz, M. (2001). Haematological profile following immunomodulation during late gestation in Buffaloes (*Bubalis Bubalus*). Pakistan Vet. J. 21 (3).

Radostits, O. M.; Gay, C. C.; Blood, D. C. and Hinchcliff, K. W. (2000). Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses, 9thed., WB Saunders. London. Pp:1414-1418,1515-1533.

Radostits, O. M.; Gay, C. C.; Hinchcliff, K. W. and Constable, P. D. (2007). Veterinary Medicine: A Textbook of the Disease of Cattle, Sheep, Pigs, Goats and Horses, 10nth ed. Saunders Elseveir. London. Pp:1735,1943.

Ramadan, A.A.; Ghoniem, A.A.; Hassan, H.M. and Youssef, A.E. (2001). Effects of β-carotene, selenium and vitamin A on in vitro polymorphonuclear leukocytic activity in peripartal buffalo (*Bubalus bubalis*). Theriogenology. 55 (3) : 693-704.

Reddy, P. G. ; Morrill, J. L.; Minocha, H. C. and Stevenson, J. S. (1987). Vitamin E is immunostimulatory in calves. J. Dairy Sci. 70 : 993-999.

Reddy, P. G.; Morrill, J. L.;Frey, R. A.;Morrill, M. B.; Minocha, H. C.;Galitzer, S. J. and Dayton, A. D. (1985) Effects of supplemental vitamin E on the immune system of calves. J. Dairy Sci. 69:164.

Rice, D. A. and Kennedy, S. (1988). Assessment of vitamin E, selenium and polyunsaturated fatty acid interactions in the aetiology of disease in the bovine. Proc Nutr. Soc 47 : 177-184.

Rice, D. A.; Blanchflower, W. J. and McMurray C. H. (1981). Reproduction of nutritional degenerative myopathy in the post ruminant calf. Vet. Rec. 109 : 161.

Robinson, J. J.; Rooke, J. A. and McEvoy, T. J. (2002). Nutrition for conception and pregnancy CAB International . Sheep Nutrition (eds M.Freer and H. Dove).

Ross, D. (1977). Oxidative killing of microorganisms by phagocytic cells. Trends Biochem. Sci. 2 : 61.

Rotruck, J. T.; Pope, A. L.; Ganther, H. E.; Swanson, A. B.; Hafeman, D. G. and Hoekstra, W. G. (1973). Selenium: Biochemical component of glutathione peroxidase. Sci. 179 : 585.

Salman, M.J. (2003). Effect of using vitamin E in increase of immunological response against sheep pox disease and their effect in protection efficacy. M.sc. Thesis, college of veterinary medicine, University of Baghdad.

Sang-Huan, O.;Myung-Ho, L. and Chang-Jo, C. (1982) Protection of phagocytic macrophages from peroxidative damage by selenium and vitamin E. Yonsei Medical J. 23 (2):101-109.

Schwartz, K. and Foltz, C. M. (1957). Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. J. Am. Chem. Soc. 79 : 3292-3293.

Scott, M. L. (1980). Advances in our understanding of vitamin E. Fed Proc 39 : 2736-2739.

Shamberger, R. J. (1983). Biochemistry of Selenium. Plenum Press, New York, Pp: 334.

Shinde, P.L.; Dass, R.S. and Garg, A.K. (2009). Effect of vitamin E and selenium supplementation on haematology, blood chemistry and thyroid hormones in male buffalo (*Bubalus bubalis*) calves. J. Anim. and Feed Sciences. 18 : 241–256.

Shinde, P.L.; Dass, R.S.; Garg, A.K. and Chaturvedi, V.K. (2007). Immune response and plasma alpha-tocopherol and selenium status of buffalo (*Bubalus bubalis*) calves supplemented with vitamin E and selenium. Asian-Austr. J. Anim. Sci. 20 : 1539-1545.

Smith, K. L.; Hogan, J. S. and Conrad, H. R. (1987). Vitamin E, selenium levels may be linked to mastitis in dairy cows. Feedstuffs, June 8 : l0.

Smith, K. L.; Hogan, J. S. and Weiss, W. P. (1997). Dietary vitamin E and selenium affect mastitis and milk quality. J. Anim. Sci. 75: 1659-1665.

Smith, K. L.; Conrad, H. R.; Amiet, B. A. and Todhunter, D. A. (1985). Incidence of environmental mastitis as influenced by vitamin E and selenium. Kiel. Milchwirtsch. Forschungsber.37: 482.

Smith, K.L.; Harrison, J. H.; Hancock, D. D.; Todhunter, D. A. and Conrad, H. R. (1984). Effect of vitamin E and selenium supplementation on incidence of clinical mastitis and duration of clinical symptoms. J. Dairy Sci. 67 : 1293.

Spears, J. W.; Harvey, R. W. and Segerson, E. C. (1986). Effects of marginal selenium deficiency and winter protein supplementation on growth, reproduction and selenium status of beef cattle. J. Anim. Sci. 63 : 586-594.

SPSS.(1996) Statistical packages for windows integrated student version.Version 13.0,SPSS Inc.,Chicago,Illinois.

Steen, A. ; Strøm, T. and Bernhoft, A. (2008). Organic selenium supplementation increased selenium concentrations in ewe and newborn lamb blood and in slaughter lamb meat compared to inorganic selenium supplementation. Acta. Vet. Scan. 50 :7.

Steiss, J. E. (1985). Effect of disulfiram in experimentally induced vitamin E deficiency myopathy in lambs. Am. J. Vet. Res. 46 : 2141–2144.

Strom, T.; Carpenter, C. and Garovoy, M. (1973). The modulating influence of cyclic nucleotides upon lymphocyte mediated cytotoxicity. J. Exp. Med. 138 : 301.

Surai, P. F. (2006). Selenium in nutrition and health, Nottingham University Press,UK. 974.

Tarn, B. K. and McCay, P. B. (1970). Reduced TPNH oxidase-catalyzed alterations of membrane phospholipids. III. Transient formation of phospholipid peroxides. J. Biol. Chem. 245 : 2295-2300.

U.S. FDA Food and Drug Administration. Direct microscopic somatic cell count, Department of Health and Human services, Public Health Service, Milk Laboratory Evaluation.

Ullrey, D. E. (1981). Selenium in the soil-plant-food chain. In: Selenium in biology and medicine, J, E, Spallholz, J- L. Martin, and H, E, Ganther (eds). Avi Publ. Co. Connecticut. Pp: 176.

Underwood, E. J. and Suttle, N. F. (1999). Selenium. In: Mineral Nutrition of Livestock. CAB International. 421–475.

Van Saun, R. J.; Herdt, T. H. and Stowe, H. D. (1989). Maternal and fetal vitamin E concentrations and selenium-vitamin E interrelationships in dairy cattle. J. Nutr. 119 : 1156.

Varley, H.; Gowenlock, A.H. and Bell, M. (1976) The tocopherols. In: Varley H (ed) Practical clinical biochemistry, vol. 2. Hormones, vitamins, drugs and poisons. Heinmann Medical, London. Pp: 222-223.

Wang, X. and Quinn, P.J. (1999). Vitamin E and its function in membranes. Prog. Lipid Res. 38 (4): 309–336.

Wastell, M. E.; Ewan, R. C.; Vorhies, M. W. and Speer, V. C. (1972).Vitamin E and Selenium for growing and finishing pigs. J. Anim. Sci. 34 : 969-973.

Weber, B.; Nickol, M. M.; Jagger, K. S. and Saelinger, C. B. (1982). Interaction of Pseudomonas exoproducts with phagcytic cell. Can. J. Microbiol. 28:679-685.

Weiss, W.P.; Colenbrander, V.F. and Cunningham, M.D. (1984) Maternal transfer and retention of supplemental selenium in neonatal calves. J. Dairy Sci. 67 (2) : Pp: 416-420.

Weiss, W. P.; Hogan, J. S.; Smith, K. L. and Hoblet, K. H. (1990). Relationships among selenium, vitamin E, and mammary gland health in commercial dairy herds. J. Dairy Sci. 73 : 381.

Welch, J. G.; Hoekstra, W. G.; Pope, A. L. and Philips, P. H. (1960). Effects of feeding fish liver oil, vitamin E and selenium to ewes upon the occurrence of muscular dystrophy in their lambs. J. Anim. Sci. 19 : 620-628.

Whanger, P. D. (2002). Selenocompounds in plants and animals and their biological significance. J. Amer. College of Nutrition. 21: 223–232.

Whanger, P. D.; Weswig, P. H.; Muth, O. H. and Oldfield, J. E. (1969). Tissue lactic dehydrogenase, glutamic-oxalacetic transaminase, and peroxidase changes of selenium-deficient myopathie lambs. J. Nutr. 99 : 331-337.

Whiet, C. L.; Caldwalader, T.K. and Hoekstra, W. G. (1989). Effects of copper and molybdenum supplements on the copper and selenium status of pregnant ewes and lambs. J. Anim. Sci. 67: 803-809.

Whitmore, C.M. (1965) A microcytic anemia of juvenile chinook salmon resulting from diets deficient in vitamin E. http://hdl.handle.net/1957/3019.

Wichtel, J. J.; Craigie, A. L.; Varela-Alvarez, H. and Williamson, N. B. (1994). The effect of intraruminal selenium pellets on growth rate, lactation, and reproductive efficiency in dairy cattle. New Zealand Vet. J. 42 : 205-210.

Wildeus, S. (2000). Current concepts in synchronization of estrus: Sheep and goats. J Anim. Sci. 77:1-14.

Wright, P. L. and Bell, M. C. (1964). Selenium-75 metabolism in the gestating ewe and fetal lamb: Effects of dietary alpha-tocopherol and selenium. J. Nutr. 84 : 49.

Wuryastuti, H.; Stowe, H. D.; Bull, R. W. and Miller, E. R. (1993). Effects of vitamin E and selenium on immune responses of peripheral blood, colostrum, and milk leukocytes of sows. J. Anim. Sci. 71 : 2464-2472.

Yokota, T.; Igarashi, K. and Uchihara, T. (2001). Delayed-onset ataxia in mice lacking alpha-tocopherol transfer protein: model for neuronal degeneration caused by chronic oxidative stress. Proc Natl. Acad. Sci. USA 98 : 1518.

الخلاصة

أجريت دراسات سريرية,دمية ومناعية لنقص فيتامين E والسلينيوم المستحدث أذ أستعملت ثمانية وعشرون نعجة عواسية وأربعة وعشرون من حملانها لذلك أستغرقت مدة الدراسة تسعة أشهر بدأت في الأول من شهر آذارلسنة 2011 وأنتهت في الأول من شهر كانون الأول لنفس السنة وتضمنت الخطوات الآتية:-

الخطوة الأولى:أختيارالحيوانات وتصنيفها:

كانت حيوانات الدراسة تعود إلى الهيئة العامة للبحوث الزراعية/وزارة الزراعة.نظام تربية الحيوانات هو من نوع النظام المكثف. أختيرت أربعون نعجة عواسية,عشرون منها تم أختيارها من قطيع متكون من مئة وعشرين نعجة بعمر ثمانية عشر شهرا غير مسفدة سابقا وغير ملقحة بلقاح Rev 1 العشرون نعجة الأخرأختيرت من قطيع لنعاج بعمر ستة وعشرين شهرا مع أكثر من ولادة سابقا وكانت ملقحة بلقاح Rev 1 .

الخطوة الثانية:أجراء توحيد الشبق:

تم التأكد من صحة أرحام النعاج قبل أجراء توحيد الشبق بأستعمال جهاز السوناربعد ذلك أستعملت أسفنجات مهبلية لأجراء توحيد الشبق.تم تقسيم النعاج أعتمادا على نوع العليقة,نوع المعاملة واللقاح إلى المجاميع كما يلي:-النعاج في المجموعة الاولى كانت بعمرستة وعشرين شهروتمت تغذيتها على عليقة النقص وكانت ملقحة بلقاح Rev1 ,النعاج في المجموعة الثانية كانت بعمر ثمانية عشر شهراوتمت تغذيتها على عليقة النقص فقط,النعاج في المجموعة الثالثة كانت بعمرستة وعشرين شهراوكانت ملقحة بلقاح Rev1 فقط والنعاج في المجموعة الرابعة كانت بعمرثمانية عشر شهراتمت تغذيتها على العليقة العادية وتركت كمجموعة سيطرة.

الخطوة الثالثة:أستحداث نقص فيتامين E والسلينيوم :

تم أستحداث نقص فيتامين E والسلينيوم بأعطاء عليقة تحتوي على زيت السمك يتركيز 3% أذ تم مزجه مع الذرة المطحونة بمقدار نصف كيلو غرام لكل حيوان في اليوم, تبن رديئ النوعية بطريقة مفتوحة وماء بطريقة مفتوحة.

الخطوة الرابعة:جمع العينات والعمل المختبري:

تم سحب عينات الدم بالتزامن مع ظهورأعراض النقص في الحملان والنعاج, سحبت عينات دم بدون مانع تخثر وفصل المصل منها لقياس مستوى فيتامين E والسلينيوم وتم أيضا قياس فعالية أنزيم creatine kinase وأنزيم aspartate aminotransferase .تم جمع دم مع مانع تخثر وتم أستماله لغرض حساب أعداد كريات الدم البيض الكلي والتفريقي ,أجراء فحص هشاشة كريات الدم الحمر وأجراء عملية البلعمة.

تم جمع البأ خلال اليوم الأول من الولادة لغرض قياس مستوى فيتامين E والسلينيوم.تم جمع الحليب بعد أنتهاءأفراز البأ لغرض قياس فيتامين E والسلينيوم وأجراء عد الخلايا الجسمية.

كانت العلامات السريرية للنقص في هذه الدراسة تتصف بشكل رئيسي بالموت المفاجئ في أربعة من الحملان من أصل اربعة عشر حمل المولودة من نعاج مجموعتي النقص.الحملان العشرة الأخرى المولودة للنعاج في مجموعتي النقص والتي لم تنفق أظهرت علامات متباينة تضمنت عدم القدرة على الرضاعة ,تقوس الظهر,ضعف,خمول,هزال ورقود.التغييرات مابعد الموت في الحملان النافقة أتصفت بالشحوب لعضلة القلب والضمور لعضلات الفخذ.العلامات السريرية في النعاج الناقصة أظهرت نقصان في وزن الجسم, أنخفاض أنتاج الحليب وفقدان الصوف فضلاعن الضعف,الخمول والرقود.

أظهر تقديرفيتامين E والسلينيوم في المصل,البأوالحليب أنخفاض في مستوى هذين العنصرين في الحيوانات الناقصة عنه في الحيوانات الصحيحة مع وجود فروقات معنوية ($P<0.05$) بين المجاميع المختلفة للنعاج والحملان.فحص البلعمة أظهر قيم أقل في حيوانات النقص عنه في الحيوانات غير الناقصة مع وجود فروقات معنوية ($P<0.05$) بين المجاميع المختلفة للنعاج والحملان.

أضهر فحص هشاشة كريات الدم الحمرأن بداية التحلل واكتماله كانت أعلى في حيوانات النقص مقارنة مع الحيوانات الغير ناقصة مع وجود فروقات معنوية ($P<0.05$) بين المجاميع المختلفة.

كذلك أظهرت النتائج أنخفاض في عددكريات الدم البيض والخلايا اللمفية في حيوانات النقص مقارنة مع الحيوانات غير الناقصة مع زيادة في عدد الخلايا المتعادلة,الحمضية والخلايا القاعدية في حيوانات النقص مقارنة مع مثيلاتها في الحيانات غير الناقصة مع وجود فروقات معنوية ($P<0.05$) بين المجاميع المختلفة.

وهناك زيادة في مستويات أنزيم ال creatine kinase وأنزيمaspartate aminotransferase في حيوانات النقص مقارنة مع الحيوانات غير الناقصة مع وجود فروقات معنوية ($P<0.05$) بين

المجاميع المختلفة أما عينات الحليب فقد أظهرت أعلى مستوى لعدد الخلايا الجسمية في نعاج النقص مقارنة مع العدد في الخلايا الجسمية في الحيوانات غير الناقصة مع وجود فروقات معنوية ($P<0.05$) بين المجاميع المختلفة.

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أستحداث نقص فيتامين E والسلينيوم في النعاج العواسية وحملانها

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We, the members of the examining committee, certify that after reading this thesis ***"Induction of vitamin E and selenium deficiency in Awassi ewes and their newborn"*** in its contents, we think it is adequate for the award of the degree of ***Doctor of Philosophy in Science of Veterinary Medicine / Internal and Preventive Veterinary Medicine.***

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Chapter one

Introduction

Chapter Two

Review of Literatures

Chapter Three Materials & Methods

Chapter Four Results and Discussion

Conclusions and Recommendations

References