



**STUDYING THE EFFECT OF ADDING SOME MINERAL SALTS ON PHYSIOLOGICAL
AND PRODUCTIVE PERFORMANCE OF GRAZING DARSHAWY EWES DURING
LATE PREGNANCY IN HADRABA VALLEY, SOUTH EGYPT**

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Article Received on 26/09/2019

Article Revised on 17/10/2019

Article Accepted on 07/11/2019

ABSTRACT

The plants dominant in the range at south desert of Egypt could not ensure a mineral balanced diet to the local animals. The aim of this study was to investigate the effect of providing ewes, during critical physiological statuses, with some minerals in either inorganic or organic form on physiological and productive performance. The mineral additives included copper, zinc, manganese and cobalt. The study was carried out in the Valley of Hadraba in – El-Shalateen – Aboramad – Halayib triangle, south east of Egypt. A total of 45 Darshawy ewes, a local sheep breed, were divided into three groups (15 ewes for each). Ewes of the first group (control) were fed normal diet. Ewes of the second group were provided with some mineral elements in the inorganic form, while ewes of the third group were provided with the same minerals in the organic form. Minerals were added to the concentrated feed mixture. The experiment lasted for a full breeding season. Data were recorded during the last three months of pregnancy. Physiological parameters included complete blood picture and electron microscopy swabs to measure the size and shape of erythrocytes. As well, cardio-thermo respiratory response, erythrocytes and leucocytes indices, antioxidant, oxidant and immune-cell-mediator (cytokine) indices, liver and kidney functions and some plasma mineral contents were assessed. It was found that using mineral additives to pregnant Darshawy ewes help in improving many physiological characteristics such as reducing cardio-thermo-respiratory responses, increasing blood O₂, increasing Ht, MCV, MCH, WBCs and secretion of T3 and cortisone. Organic minerals additive was more efficient than inorganic in increasing blood TP, plasma IgG, K, Ca, P and reproductive efficiency.

KEYWORDS: Darshawy ewes, mineral salts, immunity, blood parameters, some plasma chemicals.

INTRODUCTION

Under pasture system, animals depend on the forages to satisfy all their nutritional requirements. The performance and health of grazing livestock is dependent on the adequacy and availability of essential mineral elements from pastures. Pastures often fail to supply all needed mineral elements in adequate quantity throughout the year to grazing ruminants (Underwood and Suttle, 1999). Mineral inadequacies in forages and soils have been reported, to be the principal causes of reproductive failure and low production rates (McDowell, 1985 and 1997; McDowell and Valle, 2000 and McDowell, *et al.*, 1993). The most important mineral affecting production of grazing livestock were stated to be Ca, P, Mg, Na, S as majors and Co, Cu, I, Mn, Se and Zn as traces (Judson and McFarlane, 1998).

In Egyptian deserts, small ruminants (sheep and goats) are an important economic resource for rural people. These areas is characterized by heat stress and malnutrition that represent critical factors to animal productivity, quantity and quality, as well as natural immunity (Yasha *et al.*, 2017), especially under different physiological status. In El-Shalateen – Aboramad and Halayib triangle, seasonal variability can markedly affect the dietary intake of minerals as a result of changes in composition, stage of

growth, availability of pasture and to changes in the moisture content of the soil. Badawy (2005) and Nassar (2008) found unbalanced plasma mineral contents in camels and small ruminants that depend on pastoral areas of Egypt. Knowledge on adequate mineral supplementation to animals would form a base line data on mineral status of feed requirements for enhanced nutrition of grazing ruminants in semi-arid and arid areas of Southern Egypt. Moreover, receiving dams to adequate macro and microelements throughout pregnancy is important to optimize their capacity to manage these physiological challenges and ensure the well-being of the growing fetus, especially under heat stress.

The objective of this study was to use mineral additives either in organic or inorganic form to the Darshawy ewes raised in Hadraba valley during late pregnancy to improve the physiological hemostat including hematological, biochemical and immunological responses and reduce the adverse effects of the environmental heat stresses on their productivity.

MATERIALS AND METHODS

Experimental region

The present study was carried out during August to October 2016 in Hadraba valley, Halayib and El-Shalateen Research

Station belonging to Desert Research Center, Ministry Of Agriculture, Egypt, which lies 1300 km south east of Cairo. Hadraba valley has very good natural pasture. It lies between longitudes 36°, 52' & 36°, 45' and latitudes 22°, 59' & 23°, 59'). The air temperature (AT °C), temperature of solar radiation (TSR °C) and relative humidity (RH %) at

the level of experimental animals were monthly recorded at 07.00 and 14.00 h, simultaneously during recording the physiological responses and represented in Figure 1. A weather station (Stevens's screen) near the animals were used to measure these micro-climatic elements.

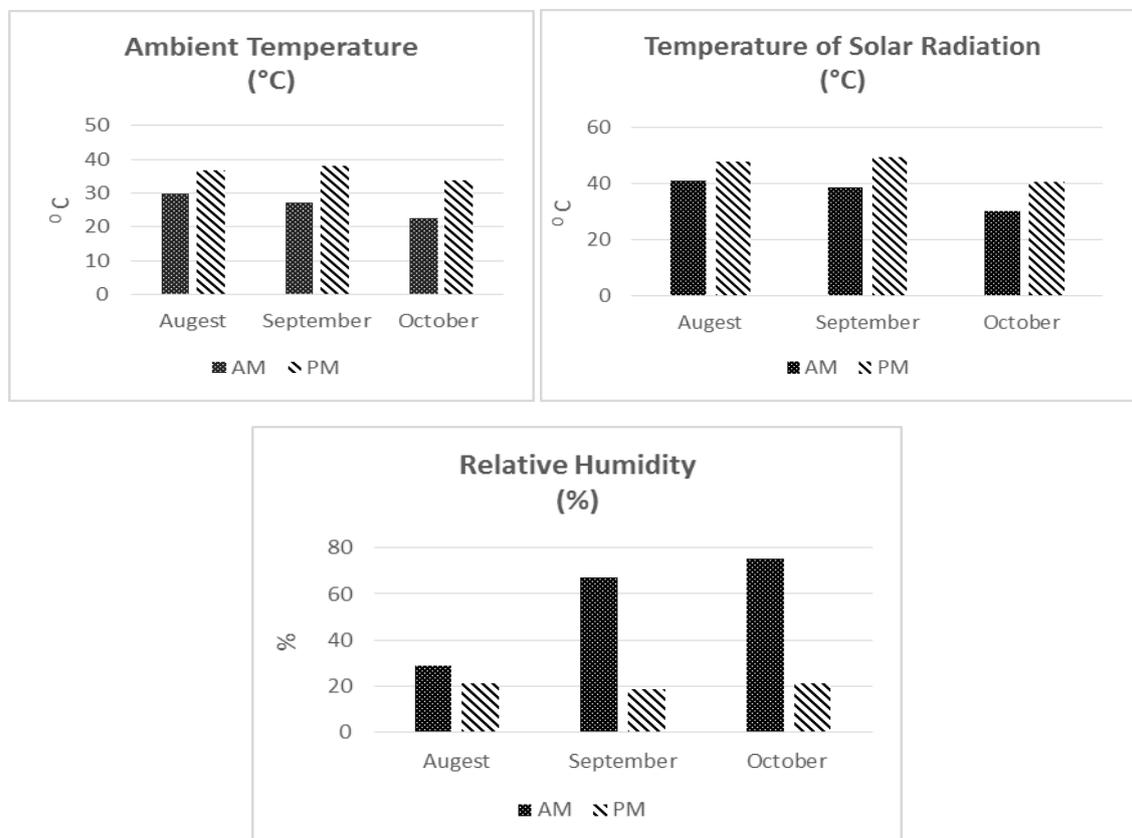


Figure 1: Microclimatic elements at the level of animals during experimental period.

Experimental animals and design

Experimental animals were kept in pens with semi-open yard. Animals went out daily to graze the natural pasture for 8 hours in two times, 8.00 am -14.00 pm and 16.00 pm - 18.00 pm. This pasture contains three main plants namely *Panicum turgidum*, *Lycium shawii* and *Acacia tortilis*. In addition, all grazing animals were supplemented with concentrate feed mixture (CFM) to cover 65% of total requirements during late pregnancy as recommended by **NRC (1985)**. The supplementary concentrate feed mixture (70% TDN and 14% CP) consisted of yellow corn 50.0%, soya bean meal 14%, wheat bran 32%, limestone 2.0%, sodium chloride 1.5% and mineral mixture 0.5%. Mineral mixture that used in formulating CFM consisted of Mn (50 g), Zn (60 g), Fe (30 g), Cu (10 g), I (1.5 g), Se (62.5 mg), Co (125 mg) and the rest CaCO₃. Supplementary feed was offered daily for ewes after grazing time (at 6 pm). Ewes of all groups were kept under the same management and hygienic conditions. Chemical analyses of grazed plants and the CFM were conducted according to **A.O.A.C. (1990)** and presented in table (1). Animals were allowed to drink water *ad lib* at 08.00 am (just before grazing), 15.00 pm at farm break and 19.00 pm after coming back from grazing. Water was obtained from the Water Condensing Station at Ras-Hadraba, and its constituents were given in table (2).

Forty five Darshawy ewes (aged 2-4 years and weighed 36.5 ± 3.5 Kg on average) were divided into 3 groups (15 ewes/group). Ewes of the first group (Control) grazed the pasture and have the CFM supplement. Ewes of the second group (Inorganic) were additionally given a salt additive mixture in inorganic form. Ewes of the third group (Organic) were given the salt additive mixture in organic form. Mineral additive in either form was added by 550 gm to 100 kg CFM. The inorganic mineral mixture was a commercial product produced by Starfarma for feed additives, Industrial region, Asyut, Egypt. Each kg of this mixture contains Mn (12.5 g), Zn (12.5 g), Fe (12.5 g), Cu (3.75 g), I (1.5 g), Se (62.5 mg), Co (125 mg) and the rest was CaCO₃. The organic mineral mixture was a combination (as a metal amino acid complex) of Zn (360 mg), Mn (200 mg), Cu (125 mg) and Co as cobalt glucoheptonate (12 mg). It was supplied by Availa@-4- (Zinpro, Eden Prairie, MN), Industrial region, Six of October City.

Natural mating season was arranged at 15th of May to 19th of June 2016 (tow estrus cycles). Data recording was performed at the last three months of pregnancy (August, September and October), one time per month. The treatment lasted for other 3 months during lactation. Reproductive performance was documented for each experimental group.

Table 1: Chemical composition of grazing plants and the concentrate feed mixture (on DM basis %).

Item	<i>Panicum turgidum</i>	<i>Lycium shawii</i>	<i>Acacia tortilis</i>	Concentrate feed mixture
DM	63.26	69.18	72.24	89.42
CP	3.85	8.46	8.14	14.07
CF	29.00	37.82	31.76	8.16
EE	3.78	1.55	3.13	3.36
Ash	8.16	7.47	7.43	4.59
OM	91.84	92.53	92.57	95.41
NFE	55.21	44.70	49.54	69.37

DM = Dry mater, CP = Crude protein, CF = Crude fiber, EE = Ether extract, OM = Organic matter, NFE = Nitrogen free extract.

Table 2: The chemical analysis of water.

Item	pH 1:2.5*	TDS	Cations (mg/l)				Anions (mg/l)			
			Na ⁺	K ⁺	Ca ⁺⁺	Mg ⁺⁺	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻²	Si ⁻⁴
Value	8.14	761.3	171.3	8.0	60.1	85.8	27.4	497.0	56.1	17.0

* Standard ratio at evaluating water by the pH meter (1 distilled water: 2.5 drinking water).

Measurements

1 - Cardio-thermo-respiratory responses

Rectal temperature (RT) was measured to the nearest 0.1 °C by using a standard clinical thermometer. Respiration rate (RR) was recorded by counting frequency of flank movements per minute. Heart rate (HR) was measured in beats per minute (bpm) by using a clinical stethoscope. Systolic and diastolic pressure were measured by using digital veterinary blood pressure monitor model Contec 08A Vit. Blood oxygen (O₂) percentage was measured by using OLED display Oximeter throughout the central ear vein.

2 - Blood picture

From each animal two blood samples were taken monthly. The first was drawn into vial containing disodium ethylene diamine tetra acetic acid (EDTA) as anticoagulant and divided into parts, the first part used for immediate determination of complete blood contents (CBC) and the other part was centrifuged at 3000 rpm for 20 minutes to obtain the blood plasma for certain biochemical analysis. The second sample was drawn in tube without anticoagulant and kept at a standstill under 6 °C to obtain the serum after two hours that was cleared by centrifugation. The serum was stored at -20 °C in glass vials until the biochemical analyses were performed.

Hemoglobin concentration (Hb g/dl) was assayed by the method of **Zijlstra (1960)**, using kits provided by Pasteur Lab. Diagnostic (Egypt). Haematocrit (Ht %) was determined according to **Hodgetts (1959)** using Wintrobe tubes. The numbers of red blood cells (RBC's 10⁶ cells/ml) and white blood cells (WBC's 10³ cells/ml) were counted using Neubauer's hemocytometer cited by **Bauer (1970)**. Electron microscopy swabs were used to measure the size and shape of erythrocytes throughout recording the surface images of nanoparticles by using Quanta FEG 250 scanning electron microscope (FEI Company, USA) available at EDRC, Desert Research Centre (DRC), Cairo. Samples were mounted on to SEM

stubs. Applied SEM conditions were: a 10.1 mm working distance, with in-lens detector with an excitation voltage of 20 kV. Blood smears were prepared for leukocytes differential count using the method described by **Cheryl et al. (1992)** and expressed as a percentage.

3 - Plasma biochemistry

Plasma contents of total protein (TP g/dl), albumin (Alb g/dl) were determined by colorimetric method using commercial kits supplied by Biodiagnostic-Egyptian Company. The concentration of globulin (Glb g/dl) and albumin to globulin (A/G) ratio were calculated. Plasma alanine transferase (ALT IU/ml), aspartate transferase (AST IU/ml), alkaline phosphatase (ALP IU/L), creatinine (Cre mg/dl) and urea (mg/dl) were measured by colorimetric method using kits supplied by Biodiagnostic-Egyptian Company. γ -glutamyl transferase (GGT IU/L) was determined by kinetic colorimetric method using kits supplied by Spectrum-diagnostics Egypt Company for Biotechnology. Triiodothyronine (T₃ ng/ml) and cortisone hormones (ng/ml) were evaluated using Elisa micro-plate reader (McCan) Model MCL-2100, China and kits from Xema Co., Ltd, Russia.

4 - Some plasma antioxidant, oxidant and immune-cell-mediators (cytokine)

Total anti-oxidant capacity (TAC) and malondialdehyde (MAD) (a lipid peroxide) were measured by colorimetric method using kits supplied by Biodiagnostic-Egyptian Company. Cytokines (interleukins 1 & 6) and tumor necrosis factor- α (TNF- α) were determined from undiluted samples using QUANTIKINE commercially available ELISA Kits (R&D Systems, Inc. 614 McKinley Place NE Minneapolis, MN 55413 USA).

Complement immune proteins including complement 3 (C₃) and complement 4 (C₄) and plasma total immunoglobulin subsets (IgG, IgM) were measured by

ELISA kits according to Abbott Laboratories instructions (Abbott Park, IL 60064 USA).

5 - Some serum mineral contents

The serum macro (Na, K, Ca and P mmol/l) and micro elements (Mg, Mn, Cu, Zn and Se) were measured by colorimetric method using commercial kits supplied by Bio-diagnostic-Egyptian Company.

6 - Reproductive efficiency

Reproductive data for ewes of each experimental group were recorded from mating to weaning their lambs.

Statistical analysis

Physiological responses were analyzed by the least square analysis of variance using the General Linear Model Procedure (SAS, 2004). The design was three-way analysis of variance with repeated measurements for thermo-respiratory responses. Meanwhile, other parameters were analyzed using two-way analysis of variance with repeated measurements.

RESULTS AND DISCUSSION

1- Cardio-thermo-respiratory responses and blood oxygen

The thermo-respiratory responses of pregnant Darshawy ewes were affected significantly by treatment, month, diurnal variation and their interactions (Table 3). Ewes exhibited the highest temperatures of rectal (RT), skin (ST) and coat (CT), in addition to the highest respiration rate (RR) during August since it was the hottest month (Fig. 3). Afternoon (14.00pm) records of all thermo-respiratory responses were higher ($P < 0.01$) than those of morning (08.00 am) due to climatic diurnal variations.

Treated ewes, in general, exhibited lower RT, ST, CT and RR than control ones. While inorganic salt helped in reducing RT, the organic salt additive was more effective in reducing ST, CT, RR and the diurnal variation of RT. The lowest blood O_2 was recorded during August and the highest was in October. This monthly trend followed that of RR. Increasing RR from August to October in an opposite trend to $AT^{\circ}C$ might be due to increasing RH% and metabolic heat production with advancing pregnancy. *Idonije et al. (2011)* explained that the stressful stages of pregnancy and early lactation resulted in a rise of high metabolic demand and in an elevating the requirements for tissue oxygen. This could be explained by the fact that 80% of fetus growth occurs in the last 2 months of pregnancy, so ewes exhibit a dramatic increase in metabolism during this period (*Cristian and Jauhianinen, 2001*). Mineral additive treatments raised blood O_2 % of ewes as compared to control ones. The increase was more in ewes received inorganic salt additive.

Heart rate (HR) in pregnant Darshawy ewes decreased towards the end of pregnancy, i.e. from August to October (Table 4). It seemed that heart rate was more influenced by hot climate than stress of heavy pregnancy.

Treatment helped to reduce ($P < 0.05$) HR, but organic salt was the more effective.

Blood pressure was not affected by month. Ewes had inorganic mineral additives showed the highest values of systolic and diastolic blood pressure either at morning or afternoon.

Changes in RT, RR and HR are the key parameters that indicate the mechanism of physiological adaptation in small ruminants (*Sejian et al., 2017*). When animals exposed to the high ambient temperature of about 40 to 44 $^{\circ}C$, RR, HR and RT increase (*Hooda and Upadhyay, 2014*). Extreme ambient conditions can negatively affect animal production and regulation of metabolic processes (*Góngora and Hernández, 2010*). In this way, many productive and reproductive parameters can be impaired, like nutrient intake, weight gain, milk production and fertility (*Malmkvist et al., 2009*). Adaptive processes to mitigate heat stress begin at conception and continue throughout gestation. The objective of adaptation in this period is to achieve successful outcome of gestation, survival of the neonate and lactation to nourish the offspring. In this experiment, thermo-respiratory responses might indicated that mineral additives, especially in organic form, could help ewes to be more adapted to both hot climatic conditions and high metabolic heat production during late pregnancy. Nevertheless, ewes received organic mineral additive showed the least levels of both T3 and cortisone at the end of pregnancy (Table 7).

2- Erythrocyte indices

Red blood cells count (RBCs), hemoglobin concentration (Hb) as well as mean corpuscular hemoglobin concentration (MCHC) decreased in treated ewes than control ones (Table 5). The decrease was more pronounced in ewes received inorganic additive. Using inorganic mineral additive resulted in increasing ($P < 0.05$) hematocrit (Ht), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values than other groups. For month effect, only RBCs count increased gradually with advancing pregnancy. *Rodger et al. (2015)* explained that plasma volume increases progressively throughout normal pregnancy resulting in hemodilution. As a result, there is a fall in Hb, Ht and RBCs count. They added that, there is usually no changes in MCV or MCHC. The present results might indicate that inorganic mineral additive augmented the stress of hemodilution.

In the control group, the Morphology of the erythrocytes by using scanning electron microscope (SEM) showed decrease in the size of these cells and their diameter which ranged from 3.3 μm to 4.1 μm (Fig. 2). Also, there were increase in the abnormal erythrocytes especially echinocytes and stomatocytes in which cells appear cup shaped with slit like central area (stoma) (Fig. 3). In case of the inorganic group, the morphology of the erythrocytes showed normal size and decrease in the

morphology abnormalities. The diameter of the cells about $3.9 \mu\text{m}$ (Figs. 4 and 5).

In the organic group, the erythrocytes showed normal size and their diameter ranged between $3.8 \mu\text{m}$ and $4.3 \mu\text{m}$. The abnormal erythrocytes still of higher ratio. The abnormal erythrocytes such as kinzoocytes appeared pinched in the center with invaginations on the membrane. The echinocytes showed regularly spaced short projections and stomatocytes were also present (Figs. 6 and 7).

The observations revealed significant poor conditions and hypoxia or Oxygen deficiency or the animals suffering from anemia and these findings were in agreement with **Han *et al.* (2005)**. The administration of the inorganic salts improve the morphology of the cells but the size still under normal. The results also showed that the administration of the organic salts improve the size of erythrocytes and the abnormal erythrocytes still present and these abnormalities were in accordance with **Swanepoel and Pretorius (2011)**.

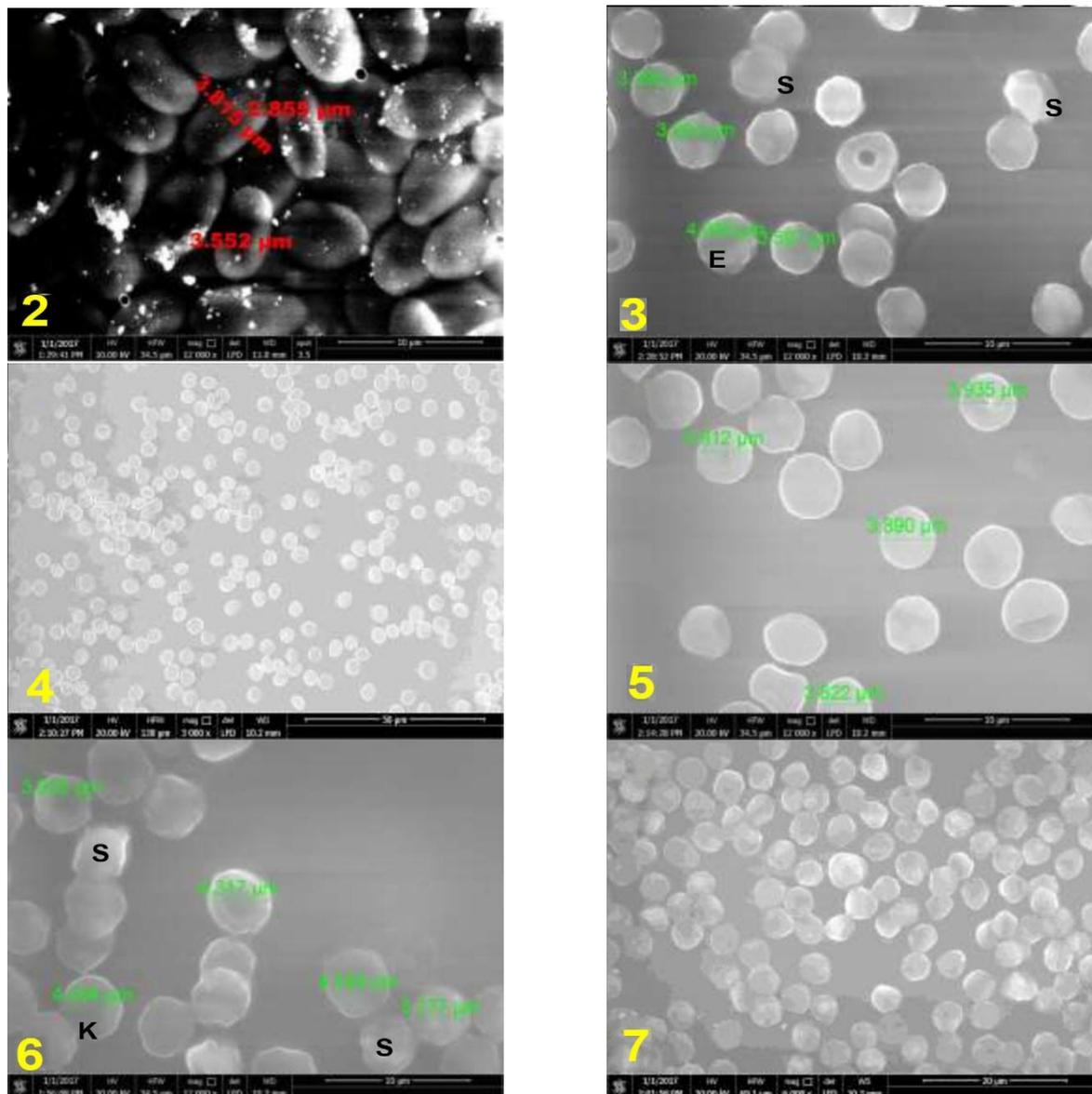


Figure 2: Scanning electron micrograph showing a decrease in size of the erythrocytes of the control individuals. (X12000).

Figure 3: Scanning electron micrograph showing a decrease in the size of erythrocytes and an increase in the abnormal cells; Echinocytes (E) and stomatocytes (S) of the control individuals. (X12000).

Figure 4: Scanning electron micrograph showing a decrease in the abnormality of erythrocytes of the inorganic salt individuals. (X3000).

Figure 5: Scanning electron micrograph showing an increase in size of the erythrocytes of the inorganic salt individuals. (X12000).

Figure 6: Scanning electron micrograph showing the normal size and abnormal morphology of erythrocytes of the organic salt individuals. Echinocytes (E), Kinzoocytes (K) and stomatocytes (S). (X12000).

Figure 7: Scanning electron micrograph showing abnormal morphology of erythrocytes of the organic salt individuals. (X6000).

3- Leucocytes indices

Addition of organic minerals additive to pregnant Darshawy ewes resulted in significant increase in WBCs count (Table 6). This increase was mainly due to the increase in lymphocytes percentage. **Cole (1990)** stated that increased lymphocytes might be attributed to stress and immune response to the environment. Inorganic mineral additive also resulted in increasing lymphocytes percentage. It can be concluded that mineral additives enhance immune capacity of ewes. However, mineral additives decreased ($P<0.05$) the percentages of monocytes and granulocytes. However, month did not affect leucocyte indices.

4- Changes in some biochemical plasma contents

a): Plasma total protein (TP) was elevated ($P<0.05$) by adding organic minerals additive, while decreased at using inorganic one (Table 7). Albumin (Al) decreased in treated ewes, while globulin (Gl) increased as compared to control. However, ewes had organic mineral additive developed higher plasma TP, Al and Gl than those had inorganic one.

b): Treatment resulted in rising secretion of both T3 and cortisone (Table 7). However, T3 increased significantly by adding organic additive, while inorganic additive raised ($P<0.05$) cortisone. This might indicate increasing basal metabolic rate as a result of mineral contents of additives. Providing trace elements was proved to be important for thyroid function. Selenium has received the most attention with respect to peripheral metabolism of thyroid hormones. Deficiencies of selenium were associated with impaired type I 5'-deiodinase activity in the liver and kidney and reduced T3 levels (**Beckett et al., 1989 and Arthur et al., 1993**). In addition, the activity of type I 5'-deiodinase was also reduced by 67 percent in zinc-deficient animals (**Kralik et al., 1996**). Also, copper deficiency enhances the effect of hypothyroidism (**Aurthor et al. 1996**).

Rising cortisone in late pregnancy is of vital importance. **Fowden et al. (1998)** explained that umbilical glucose uptake is reduced in fetal sheep during late gestation. When fetal cortisone concentrations are raised, irrespective of whether this increment cortisone is exogenous or endogenous in origin, fetal glucose levels increased despite the reduced umbilical glucose uptake, which suggests that hepatic glycogenesis was activated in these circumstances.

c): Results of liver enzymes especially ALT, AST and AIP (Table 8) indicated that liver exerted more efforts when receiving mineral additive. However, changes in γ -glutamyl transferase (GGT) was in reverse direction to other liver enzymes.

Concentrations of plasma enzymes ALT, AST and GGT are those conventionally used for diagnosing hepatic damage. Most plasma enzymes come from different tissues of animal, and its level had a relation with

metabolism and functional status of certain organs. Body's ability to adjust and adapt depends on the function of tissues and organs largely (**Shi-Gang et al., 2010**). Total proteins and its components increased toward the end of pregnancy. Coincidentally, AST and ALT increased with advancing pregnancy. Growth of fetus needs more efforts from liver for amino acids (AA) incorporation in different tissues. **Antunovic et al. (2002)** stated that increased fetal growth needs utilization of AA from the maternal serum protein. The present results indicated that mineral additive helped in liver activation that might reflect on fetus growth.

d): Inorganic mineral additive resulted in the highest blood urea followed by that of ewes received organic mineral additive. Ewes received inorganic mineral additives increased obviously blood urea with advancing pregnancy. They also showed the highest level of serum cortisone (Table 7) especially at the end of gestation. **Silanikove (2000)** stated that high blood urea could be due to increase cortisone level affecting the catabolism of protein in the body. Also, the high level of serum urea had been attributed to excessive tissues protein catabolism associated with protein deficiency (**Oduye and Adedevon, 1976**). The higher values of blood urea over the gestation period were reported by **El-Sherif and Assad (2001)** in Barki ewes and **Durak and Altinek (2006)** in Chios ewes.

Yokus et al. (2006) stated that late pregnancy resulting in increased blood volume that induce increase in glomerular filtration which responsible for increased blood urea. However, in the present study blood urea on average did not change with month of pregnancy.

Creatinine did not affected by treatment but decreased with advancing pregnancy. This was beneficial for ewes since high creatinine is indicative of poor protein and amino acid metabolism that can lead to impaired renal function and cardiac infarction (**Gray and Howarra, 1980**). It seemed that kidney function was little affected by stress of pregnancy in ewes of control group and those received organic mineral additive, while exerted more effort with inorganic mineral additive.

5- Changes in some plasma antioxidant, oxidant and immune-cell-mediators (cytokine)

All anti-oxidant and immune-cell-mediator parameters were not affected by advancing pregnancy (Table 9). Organic mineral additive increased ($P<0.05$) total antioxidant capacity (TAC), malondialdehyde (MDA) and IL-6 of pregnant Darshawy ewes. Meanwhile, inorganic mineral additive led to significant increase in TAC, and IL-6 but did not affect MDA.

Pregnancy is known to be stressful on organisms, which accelerates the production of reactive oxygen species (ROS) and oxidative stress (**Gorecka et al., 2002**). The ROS are normally neutralized by enzymatic and mineral supplementation systems of the animals. The imbalance

between the rate of ROS production and their neutralization leads to the oxidative stress. In the present study, increased TAC, MDA and IL-6 by mineral additives seemed to help pregnant Darshawy ewes to resist free radicals.

Both IL-1 and TNF- α values decreased by adding organic and inorganic mineral additives. The decrease was more pronounced by inorganic mineral additive. It well established that ROS exert a biphasic effect during pregnancy and parturition and at adequate levels are fundamental for many physiological events to occur such as embryo implantation (**Mutinati et al., 2013**). Accordingly, the reduction in both IL-6 and TNF- α by mineral additives might be of beneficial effect for ewes to complete pregnancy and lambs delivery.

Cytokines are soluble mediators and assist in the regulation of the immune response, and are generally produced by macrophages, with TNF- α and IL-1 being the first to appear in an inflammatory response (**Gengelbach and Spears, 1998**). **Waller et al. (2003)** verified that TNF- α and IL-1 participates in neutrophil chemotactic activity during an inflammatory process. In addition, TNF- α is considered a pro-inflammatory cytokines like that made by cells such as macrophages and the Type 1 T helper cells (**Trinchieri, 2007**). Furthermore, cytokine IL-1 was first defined as a polypeptide derived from mononuclear phagocytes that enhanced T-cell response to antigens. It also acts as a mediator of the host inflammatory response in natural immunity (**Dinarelo, 1996**). Moreover, it increase the expression of adhesion factors on endothelial cells to enable the transmigration of leukocytes; the cells that fight pathogens; to sites of infection (**Nicklin et al., 2000**). In the present experiment, the decrease in IL-1 and TNF- α were higher by inorganic mineral additive than by organic one that might be so harmful to pregnant ewes.

6- Changes in some immune protein antibody

Ewes received organic mineral additive showed obvious increase in IgG. Both treatments resulted in a decrease of IgM, C3 and C4 (Table 10).

These four parameters showed sharp decrease in the last month of gestation (October). **Fisher (1980)** identified that the maternal immunity should be transferred in utero to their fetuses to be born protected against the microorganisms. Placental barriers do not allow to pass immunoglobulins from dams to the neonates in ruminants (**Khan and Khan, 1996**). Lambs depend entirely on antibodies received via colostrum (**Klobasa and Werhahn, 1989**). The IgG in serum of ewes is the crucial source for colostrum and newborn young. Pregnant cows supplemented with organic minerals increased concentrations of IgG in colostrum and plasma (**Rodinova et al., 2008**). **Rock et al. (2001)** supplemented ewes with Se from 56 day of gestation until lambing and noted increased IgG absorption in their

lambs. They added that mineral supplementation to ewes in late pregnancy could result in increased colostrum Se and lamb serum IgG concentration that provide protection against diarrhea which causes lambs' deaths. Accordingly, results of the present study confirm the beneficial effect of using organic mineral additive.

7- Some plasma electrolytes

a) Macro elements

Sodium (Na) did not affected either by treatment or advancing pregnancy. Ewes had organic mineral additive developed the highest ($P < 0.05$) values of potassium (K), calcium (Ca) and phosphorus (P) (Table 11). Only K that was affected by month, where it increased ($P < 0.05$) in September than the other two months.

Minerals are of great importance to many aspects of health and normal physiological functions. Mineral constitute about 4% to 5% of body weight out of which, 50% calcium and 25% is phosphorous (**King, 2000**). The electrolytes are known to regulate osmotic pressure, maintain membrane potentials and acid base balance besides transmit nerve impulses. Sodium and potassium deficiency affect the tubes of kidney resulting in inability to concentrate urine (**Latimer et al., 2004**). Moreover, minerals have been found to be very critical in ruminants' growth and productivity (**Underwood, 1981**). Minerals required for proper functioning of ruminants' body tissues and prevent diseases as a negative effect of their deficiency (**Swecker et al., 1995**). Minerals involve in the defense system against free radicals that damage the biological system through the formation of the metal-enzymes which include glutathione peroxidase, catalase and superoxide dismutase (**McDowell, 2002**). For all previous importance of minerals, using organic mineral additive is of crucial benefit to pregnant ewes.

b) Micro elements

Micro elements (Mg, Mn and Cu) were not affected by treatment, whereas Zn and Se increased ($P < 0.05$) with the use of both inorganic and organic additives (Table 12). All micro elements were not affected by month of pregnancy. Iron (Fe) did not respond to any source of variation.

Micro elements play different roles in the body among which, participation in the construction of the body and regulation of its function especially in bone construction, transport of oxygen, regulation of blood sugar, as a cofactor for the enzyme activity, regulation of chemical reaction, protection of cell from oxidative damage and regulation of immune system function (**Blumfeld et al., 2013**).

Pregnancy presents a considerable stress to trace mineral homeostasis in mammals (**Mills and Davies, 1979**). During pregnancy, increased physiological changes to support body metabolism in the mother and growing fetus lead to an increase need for micronutrients (**Blumfeld et al., 2013 and King, 2000**). Moreover,

substantial losses of body minerals occur during pregnancy and lactation (Elnageeb and Adelatif, 2010). A significant decrease in plasma zinc was reported in the she camel at the end of pregnancy (EL-Tohamy *et al.*, 1986), due to an active transfer to the foetus in the last part of gestation. Therefore, it is of great importance to ensure whether mother receive sufficient macro and micro nutrients prior to and during pregnancy (King, 2000 and Berti *et al.*, 2011). Micro nutrient deficiency both during fertilization and pregnancy leads to some increase risk which include anemia, pregnancy-induced hypertension, fetal growth restriction, increase labor complications and maternal and fetal mortality (King, 2000 and Cheesbrough, 2010).

Zinc is an essential component of over 200 enzyme systems involved in metabolism of carbohydrate, protein and nucleic acid metabolism, epithelial tissue integrity, cell repair and division, vitamin A and E transport and their utilization. In addition, Zn plays a major role in the immune system and certain reproductive hormones (Capuco *et al.*, 1990). Zinc has also been shown to increase plasma β -carotene level which is correlated to improvement in conception rates and embryonic development (Short and Adams, 1988). A severe Zn deficiency in cattle results in impaired reproduction (Spears and Kegley, 2002). Zinc deficiency has been observed in ruminants fed on deficient feedstuffs (Sharma and Joshi, 2005). Organic form of Zn (Zn propionate) showed a better response in improving semen quality (Kumar *et al.*, 2006).

Selenium is a mineral with an antioxidant function, capable of reducing the production of free radicals through the activation of the enzyme glutathione peroxidase (Shankar and Prasad, 1998). Therefore, studies with selenium supplementation demonstrated a reduction in the incidence of mastitis and somatic cell counts in cattle (Overton and Yasui, 2014), as well as increased erythrocyte and neutrophil production in the blood stream (Morgante *et al.*, 1999). Travnicek *et al.* (2007) explained that serum Se dropped in concentration with pregnancy and lactation stress in last third of pregnancy and in the first week after parturition. The positive effect of supplementation of selenium to ewes was reflected in its higher concentration in the blood serum of born lambs. The effect of selenium bound in Chlorella biomass was higher than that of selenium in the inorganic form.

8- Reproductive performance and efficiency

Reproductive performance included some parameters presented in Table (13). Treatment did not affect conception rate, lambing rate, litter size at birth. However, both type of mineral additives improved lambs' viability and weaning weight. This reflected on reproductive efficiency (kg weaned per ewe joined) that recorded higher values (14.33 and 14.54 for inorganic and organic mineral additive, respectively) than that found in control group (10.71). This indicated that

mineral additive was of great importance to achieve the best productivity of Darshawy ewes grazing natural pasture at Hadraba valley in Halayib - Aboramad – El-Shalateen triangle at south east of Egypt.

It could be concluded that using mineral additives to pregnant Darshawy ewes helped in improving many physiological characteristics such as reducing cardio-thermo-respiratory responses, increasing blood O₂, increasing Ht, MCV, MCH, WBCs and secretion of T3 and cortisone. Organic mineral additive was more efficient than inorganic in increasing blood TP, plasma IgG, K, Ca and P. Finally, organic additive achieved better reproductive efficiency as ewes received it had better litter size at birth and weaning and higher kg weaned per ewe joined.

The present study suggested that deficiency of minerals is not the main case of the abnormality in the blood of animals in this area and there are other factors affect the animals in this area and this need further studies.

Table (3): Average values of thermo-respiratory responses of pregnant Darshawy ewes as affected by different forms of mineral additives.

Parameters	Month (M)	Treatments (Tr)						Overall means	± Standard Error			
		Control		Inorganic		Organic			Tr	M	DV	Tr*M
		am	pm	am	pm	am	Pm					
Rectal Temperature (°C)	Aug	39.30	40.24	38.94	39.08	38.18	39.80	39.26 ^a	0.05 *	0.05 *	0.04 *	0.08 *
	Sep	38.86	40.10	38.10	39.24	38.58	39.32	39.03 ^b				
	Oct	38.70	39.70	38.08	38.92	38.14	39.38	38.82 ^c				
	Overall means	38.95 ^C	40.01 ^A	38.37 ^D	39.08 ^C	38.30 ^D	39.50 ^B	39.04				
	Overall means	39.48 ^A		38.73 ^C		38.90 ^B						
Skin Temperature (°C)	Aug	36.30	37.36	35.90	36.82	35.96	36.78	36.52 ^a	0.06 *	0.06 *	0.05 *	0.12 *
	Sep	35.82	36.28	35.70	35.84	34.96	35.76	35.73 ^c				
	Oct	35.98	36.78	36.58	36.72	35.02	36.26	36.22 ^b				
	Overall means	36.03 ^C	36.81 ^A	36.06 ^C	36.46 ^B	35.31 ^D	36.27 ^{BC}	36.16				
	Overall means	36.42 ^A		36.26 ^A		35.79 ^B						
Coat Temperature (°C)	Aug	35.60	36.84	36.26	36.82	34.94	36.06	36.09 ^a	0.09 *	0.09 *	0.07 *	0.15 *
	Sep	34.94	36.16	35.34	35.82	35.84	36.10	35.70 ^b				
	Oct	34.94	36.12	35.70	36.20	34.72	35.58	35.51 ^b				
	Overall means	35.16 ^C	36.37 ^A	35.77 ^B	36.22 ^A	35.17 ^C	35.91 ^B	35.77				
	Overall means	35.77 ^{AB}		35.99 ^A		35.54 ^B						
Respiration Rate (resp./min)	Aug	25.20	55.60	18.40	34.80	29.20	40.80	34.00 ^a	0.71 *	0.71 *	0.58 *	1.24 *
	Sep	30.80	44.80	20.80	39.60	16.00	30.00	30.33 ^b				
	Oct	21.20	36.00	30.80	43.20	17.60	30.80	29.93 ^b				
	Overall means	25.73 ^D	45.47 ^A	23.33 ^{DE}	39.20 ^B	20.93 ^E	33.86 ^C	31.42				
	Overall means	35.60 ^A		31.27 ^B		27.40 ^C						
O ₂ (%)	Aug	90.80	94.00	91.80	97.00	90.20	90.60	92.40 ^b	0.37 *	0.37 *	0.30 *	0.65 *
	Sep	88.20	97.80	91.20	96.00	93.60	97.00	93.97 ^a				
	Oct	88.00	93.20	96.40	98.20	93.20	99.20	94.70 ^a				
	Overall means	89.00 ^D	95.00 ^B	93.13 ^C	97.07 ^A	92.33 ^C	95.60 ^{AB}	93.69				
	Overall means	92.00 ^C		95.10 ^A		93.97 ^B						

Means with different letters are statistically different ($P < 0.05$). A, B, C = Values with different letters on the same row differ at ($P < 0.05$), a, b = Values with different letters on the same column differ at ($P < 0.05$). NS: Non significant. * Significant. DV: Diurnal variation (am and pm). O₂: Blood oxygen percentage.

Table (4): Average values of cardiac responses of pregnant Darshawy ewes as affected by different forms of mineral additives.

Parameters	Month (M)	Treatments (Tr)						Overall means	± Standard Error			
		Control		Inorganic		Organic			Tr	M	DV	Tr*M
		am	pm	am	pm	am	pm					
Heart Rate (resp./min)	Aug	73.60	97.20	62.40	73.00	66.80	78.40	75.23 ^a	0.86 *	0.86 *	0.70 *	1.50 *
	Sep	67.20	87.20	75.20	91.60	60.40	78.40	76.67 ^a				
	Oct	66.00	92.80	64.80	73.20	58.80	73.60	71.53 ^b				
	Overall means	68.93 ^C	92.40 ^A	67.47 ^C	79.27 ^B	62.00 ^D	76.80 ^B	74.48				
	Overall means	80.67 ^A		73.37 ^B		69.40 ^C						
Systolic Blood Pressure (mm/Hg)	Aug	65.00	77.60	86.00	89.60	66.00	85.80	78.33 ^{NS}	0.92 *	0.92 ^{NS}	0.75 *	1.60 *
	Sep	76.20	87.00	90.20	91.40	65.20	70.00	80.00 ^{NS}				
	Oct	62.60	88.60	85.80	92.00	68.00	75.20	78.70 ^{NS}				
	Overall means	67.93 ^D	84.40 ^B	87.33 ^B	91.00 ^A	66.40 ^D	77.00 ^C	79.01				
	Overall means	76.17 ^B		89.17 ^A		71.70 ^C						
Diastolic Blood Pressure (mm/Hg)	Aug	44.00	52.00	57.00	59.40	43.60	60.00	52.67 ^{NS}	1.01 *	1.01 ^{NS}	0.82*	1.74 *
	Sep	42.00	55.60	52.40	60.00	50.20	52.00	52.03 ^{NS}				
	Oct	41.80	57.00	58.80	68.00	43.40	50.80	53.30 ^{NS}				
	Overall means	42.60 ^C	54.87 ^B	56.07 ^B	62.47 ^A	45.73 ^C	54.27 ^B	52.67				
	Overall means	48.73 ^B		59.27 ^A		50.00 ^B						

Means with different letters are statistically different ($P < 0.05$). A, B, C = Values with different letters on the same row differ at ($P < 0.05$), a, b = Values with different letters on the same column differ at ($P < 0.05$). NS: Non significant. DV: Diurnal variation (am and pm). * Significant.

Table (5): Average values of erythrocyte indices in pregnant Darshawy ewes as affected by different forms of mineral additives.

Parameters	Month (M)	Treatments (Tr)			Overall means	± Standard Error		
		Control	Inorganic	Organic		M	Tr	Tr*M
RBCs 10 ⁶ /mm	Aug	13.50	8.83	11.40	11.24 ^b	0.34 *	0.34 *	0.60 *
	Sep	14.04	9.89	12.12	12.01 ^{ab}			
	Oct	15.08	9.86	12.09	12.34 ^a			
	Overall means	14.21 ^A	9.53 ^C	11.87 ^B	11.87			
Hb g/dl	Aug	17.10	14.83	15.98	15.97 ^{NS}	0.30 ^{NS}	0.30 *	0.52 *
	Sep	18.25	14.63	15.98	16.28 ^{NS}			
	Oct	18.35	14.60	16.15	16.37 ^{NS}			
	Overall means	17.90 ^A	14.68 ^C	16.03 ^B	16.21			
Ht %	Aug	24.55	33.55	26.08	28.06 ^{NS}	0.70 ^{NS}	0.70 *	1.21 *
	Sep	25.93	33.48	28.05	29.15 ^{NS}			
	Oct	25.63	32.13	26.33	28.03 ^{NS}			
	Overall means	25.37 ^B	33.05 ^A	26.82 ^B	28.41			
MCV fl=liter× 10 ⁻¹⁵	Aug	18.47	38.54	22.94	26.65 ^{NS}	0.98 ^{NS}	0.98 *	1.71 *
	Sep	18.48	33.90	23.42	25.27 ^{NS}			
	Oct	17.02	32.89	22.07	23.99 ^{NS}			
	Overall means	17.99 ^C	35.11 ^A	22.81 ^B	25.30			
MCH Pg=10 ⁻¹² g	Aug	12.75	17.17	14.04	14.65 ^{NS}	0.60 ^{NS}	0.60 *	1.04 *
	Sep	13.05	14.79	13.39	13.74 ^{NS}			
	Oct	12.22	14.95	13.58	13.58 ^{NS}			
	Overall means	12.67 ^B	15.64 ^A	13.67 ^B	13.99			
MCHC %	Aug	70.09	44.25	61.47	58.60 ^{NS}	2.03 ^{NS}	2.03 *	3.52 *
	Sep	71.24	43.79	59.22	58.08 ^{NS}			
	Oct	71.67	45.46	61.47	59.53 ^{NS}			
	Overall means	71.00 ^A	44.50 ^C	60.72 ^B	58.74			

Means with different letters are statistically different (P<0.05). A, B, C= Values with different letters on the same row differ at (P<0.05), a, b = Values with different letters on the same column differ at (P<0.05). NS: Non significant. * Significant RBCs: erythrocytes cells, Ht: hematocrit, Hb: hemoglobin, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration,

Table (6): Average values of leucocyte indices in pregnant Darshawy ewes as affected by different forms of mineral additives.

Parameter	Month (M)	Treatments (Tr)			Overall means	± Standard Error		
		Control	Inorganic	Organic		M	Tr	Tr*M
WBCs 10 ³ Cells/ mm ³	Aug	9.98	13.11	20.30	14.46 ^{NS}	1.17 ^{NS}	1.17 *	2.03 *
	Sep	12.63	15.54	23.27	17.15 ^{NS}			
	Oct	12.42	12.24	24.91	16.52 ^{NS}			
	Overall means	11.68 ^B	13.63 ^B	22.83 ^A	16.04			
Lymphocytes %	Aug	68.47	76.02	76.11	73.53 ^{NS}	1.64 ^{NS}	1.64 *	2.85 *
	Sep	71.40	81.65	79.35	77.47 ^{NS}			
	Oct	65.26	76.08	83.26	74.87 ^{NS}			
	Overall means	68.38 ^B	77.92 ^A	79.57 ^A	75.29			
Monocytes %	Aug	15.34	14.91	15.50	15.25 ^{NS}	0.82 ^{NS}	0.82 *	1.42 *
	Sep	15.82	12.43	12.06	13.44 ^{NS}			
	Oct	18.94	16.15	11.04	15.38 ^{NS}			
	Overall means	16.70 ^A	14.50 ^{AB}	12.87 ^B	14.69			
Granulocytes %	Aug	16.19	9.08	8.39	11.22 ^{NS}	1.07 ^{NS}	1.07 *	1.86 *
	Sep	12.78	5.92	8.60	9.10 ^{NS}			
	Oct	15.80	7.77	5.71	9.76 ^{NS}			
	Overall means	14.92 ^A	7.59 ^B	7.57 ^B	10.03			

Means with different letters are statistically different (P<0.05). A, B= Values with different letters on the same row differ at (P<0.05), NS: Non significant. * Significant. WBCs: White blood cells.

Table (7): Average values of plasma proteins, T3 and cortisone in pregnant Darshawy ewes fed on different forms of mineral additives.

Parameters	Month (M)	Treatments (Tr)			Overall means	Standard Error		
		Control	Inorganic	Organic		M	Tr	Tr*M
Total Protein (g/dl)	Aug	6.09	5.69	6.47	6.09 ^{NS}	0.13 ^{NS}	0.13 *	0.23 *
	Sep	5.77	6.19	5.62	5.86 ^{NS}			
	Oct	6.17	5.11	6.30	5.86 ^{NS}			
	Overall means	6.01 ^{AB}	5.66 ^B	6.13 ^A	5.94			
Albumin (g/dl)	Aug	3.20	3.66	3.15	3.34 ^{ab}	0.12 *	0.12 *	0.21 *
	Sep	3.87	3.50	3.40	3.59 ^a			
	Oct	3.78	2.01	3.31	3.03 ^b			
	Overall means	3.62 ^A	3.06 ^B	3.29 ^{AB}	3.32			
Globulins (g/dl)	Aug	2.89	2.03	3.32	2.75 ^a	0.16 *	0.16 ^{NS}	0.27 *
	Sep	1.90	2.69	2.22	2.27 ^b			
	Oct	2.40	3.10	2.99	2.83 ^a			
	Overall means	2.40 ^{NS}	2.61 ^{NS}	2.84 ^{NS}	2.61			
A/G ratio (%)	Aug	1.14	1.82	1.01	1.33 ^b	0.12 *	0.51 ^{NS}	0.21*
	Sep	2.18	1.41	1.55	1.71 ^a			
	Oct	1.62	0.67	1.34	1.21 ^b			
	Overall means	1.65 ^{NS}	1.30 ^{NS}	1.30 ^{NS}	1.42			
T3 (ng/dl)	Aug	0.41	0.55	0.69	0.55 ^{NS}	0.05 ^{NS}	0.05 *	0.08 *
	Sep	0.41	0.64	0.81	0.62 ^{NS}			
	Oct	0.45	0.62	0.84	0.64 ^{NS}			
	Overall means	0.42 ^C	0.60 ^B	0.77 ^A	0.60			
Cortisone (ng/dl)	Aug	1.09	1.95	1.41	1.49 ^b	0.15 *	0.15 *	0.26 *
	Sep	1.26	2.02	0.87	1.39 ^b			
	Oct	2.79	3.58	2.97	3.11 ^a			
	Overall means	1.71 ^B	2.52 ^A	1.75 ^B	1.99			

Means with different letters are statistically different ($P < 0.05$). A, B, C= Values with different letters on the same row differ at ($P < 0.05$), a, b = Values with different letters on the same column differ at ($P < 0.05$). NS: Non significant. * Significant. A/G ratio: Albumin/Globulin ratio. T3: Triiodothyronine.

Table (8): Average values of liver and kidney functions' indices in pregnant Darshawy ewes as affected by different forms of mineral additives.

Parameters	Month (M)	Treatments (Tr)			Overall means	Standard Error		
		Control	Inorganic	Organic		M	Tr	Tr*M
ALT (IU/L)	Aug	61.48	79.79	64.92	68.73 ^{NS}	2.66 ^{NS}	2.65 *	4.60 *
	Sep	62.10	75.02	79.34	72.16 ^{NS}			
	Oct	76.23	74.68	72.30	74.41 ^{NS}			
	Overall means	66.61 ^B	76.50 ^A	72.19 ^{AB}	71.76			
AST (IU/L)	Aug	43.39	60.40	62.00	55.26 ^b	1.70 *	1.70 *	2.95 *
	Sep	57.13	52.21	66.29	58.54 ^{ab}			
	Oct	69.48	55.15	57.21	60.61 ^a			
	Overall means	56.67 ^B	55.92 ^{AB}	61.83 ^A	58.14			
GGT (IU/L)	Aug	47.47	47.47	47.47	47.46 ^a	1.35 *	1.35 *	2.35 *
	Sep	40.69	40.55	75.17	27.76 ^b			
	Oct	29.40	29.49	29.61	29.50 ^b			
	Overall means	39.19 ^A	39.17 ^A	27.63 ^B	34.91			
ALP (U/L)	Aug	71.90	63.22	87.60	74.24 ^{ab}	3.19 *	3.19 *	5.54 *
	Sep	62.66	73.46	93.40	76.51 ^b			
	Oct	63.00	58.86	78.00	66.62 ^a			
	Overall means	65.85 ^B	65.18 ^B	86.33 ^A	72.46			
Urea Mg/dl	Aug	42.00	48.80	44.40	45.07 ^{NS}	1.76 ^{NS}	1.76 *	3.05 *
	Sep	38.00	52.40	46.80	45.73 ^{NS}			
	Oct	36.00	59.80	43.60	46.47 ^{NS}			
	Overall means	38.67 ^C	53.67 ^A	44.93 ^B	45.76			
Creatinine Mg/dl	Aug	1.88	2.42	2.58	2.29 ^a	0.15 *	0.15 ^{NS}	0.26 *
	Sep	2.16	2.36	2.86	2.46 ^a			
	Oct	1.96	2.32	1.40	1.89 ^b			
	Overall means	2.00 ^{NS}	2.37 ^{NS}	2.28 ^{NS}	2.22			

Means with different letters are statistically different ($P < 0.05$). A, B, C = Values with different letters on the same row differ at ($P < 0.05$), a, b = Values with different letters on the same column differ at ($P < 0.05$). NS: Non significant. * Significant. ALT: Alanine transferase. AST: Aspartate transferase. GGT: γ -glutamyl transferase. ALP: Alkaline phosphatase.

Table (9): Average values of antioxidant, oxidant and immune-cell-mediator indices in pregnant Darshawy ewes as affected by different forms of mineral additives.

Parameters	Month (M)	Treatments (Tr)			Overall means	Standard Error		
		Control	Inorganic	Organic		M	Tr	Tr*M
TAC nmol/ml	Aug	0.42	0.63	0.76	0.60 ^{NS}	0.03 ^{NS}	3.19 *	0.06 *
	Sep	0.46	0.61	0.64	0.57 ^{NS}			
	Oct	0.48	0.68	0.72	0.62 ^{NS}			
	Overall means	0.45 ^B	0.64 ^A	0.71 ^A	0.60			
MDA mM/L	Aug	12.80	12.44	13.62	12.95 ^{NS}	0.33 ^{NS}	0.33 *	0.57 ^{NS}
	Sep	12.94	12.34	12.70	12.66 ^{NS}			
	Oct	12.50	11.70	13.66	12.62 ^{NS}			
	Overall means	12.75 ^{AB}	12.16 ^B	13.33 ^A	12.74			
IL-1 Pg/ml	Aug	118.40	76.20	97.60	97.40 ^{NS}	5.16 ^{NS}	5.16 *	8.95 *
	Sep	120.20	52.20	80.80	84.40 ^{NS}			
	Oct	110.00	63.60	90.60	88.07 ^{NS}			
	Overall means	116.20 ^A	64.00 ^C	89.67 ^B	89.96			
IL-6 Pg/ml	Aug	9.42	11.64	13.06	11.37 ^{NS}	0.67 ^{NS}	0.76 *	1.16 *
	Sep	7.32	15.76	11.66	11.58 ^{NS}			
	Oct	8.36	14.88	12.54	11.93 ^{NS}			
	Overall means	8.37 ^B	14.09 ^A	12.42 ^A	11.63			
TNF- α Pg/ml	Aug	12.48	8.62	10.76	10.74 ^{NS}	0.51 ^{NS}	0.51 *	0.89 *
	Sep	13.02	6.22	9.08	9.44 ^{NS}			
	Oct	12.00	7.36	10.06	9.80 ^{NS}			
	Overall means	12.50 ^A	7.40 ^C	9.97 ^B	10.00			

Means with different letters are statistically different ($P < 0.05$). A, B, C= Values with different letters on the same row differ at ($P < 0.05$), NS: Non significant. * : Significant. TAC: Total antioxidant capacity. MAD: malondialdehyde. IL-1: Interleukin 1. IL-6: Interleukin 6. TNF- α : Tumor necrosis factor alpha.

Table (10): Average values of Immune protein antibody indices in pregnant Darshawy ewes as affected by different forms of mineral additives.

Parameter	Month (M)	Treatments (Tr)			Overall means	Standard Error		
		Control	Inorganic	Organic		M	Tr	Tr*M
IgG mg/ml	Aug	17.45	12.13	27.00	18.86 ^b	1.27*	1.27 *	2.21 *
	Sep	26.90	22.10	25.95	24.98 ^a			
	Oct	6.98	6.18	4.39	5.85 ^c			
	Overall means	17.11 ^{AB}	13.47 ^B	19.11 ^A	16.56			
IgM mg/ml	Aug	3.01	2.84	3.52	3.12 ^{NS}	0.82 ^{NS}	0.82 *	1.42 *
	Sep	7.15	0.77	1.76	3.22 ^{NS}			
	Oct	1.44	0.28	1.09	0.93 ^{NS}			
	Overall means	3.87 ^A	1.30 ^B	2.12 ^{AB}	2.43			
C3 mg/ml	Aug	8.33	2.19	9.18	6.57 ^b	0.52 *	0.52 *	0.90 *
	Sep	11.42	5.18	8.08	8.23 ^a			
	Oct	1.67	0.54	0.53	0.91 ^c			
	Overall means	7.14 ^A	2.64 ^B	5.93 ^A	5.24			
C4 mg/ml	Aug	27.02	26.57	7.14	20.24 ^a	3.03 *	3.03 *	5.25 *
	Sep	16.97	17.47	7.40	13.94 ^a			
	Oct	5.15	1.95	2.24	3.11 ^b			
	Overall means	16.38 ^A	15.33 ^A	5.59 ^B	12.43			

Means with different letters are statistically different ($P < 0.05$). A, B = Values with different letters on the same row differ at ($P < 0.05$), a, b = Values with different letters on the same column differ at ($P < 0.05$). NS: Non significant. * : Significant. IgG: Immunoglobulin G. IgM: Immunoglobulin M. C3: Complement 3. C4: Complement 4.

Table (11): Average values of some serum macro elements in pregnant Darshawy ewes as affected by different forms of mineral additives.

Parameters	Month (M)	Treatments (Tr)			Overall means	Standard Error		
		Control	Inorganic	Organic		M	Tr	Tr*M
Na mmol/l	Aug	146.12	160.04	147.04	151.07 ^{NS}	2.78 ^{NS}	2.78 ^{NS}	4.82 ^{NS}
	Sep	149.78	150.58	155.76	152.04 ^{NS}			
	Oct	144.36	147.66	151.78	147.93 ^{NS}			
	Overall mean	146.75 ^{NS}	152.76 ^{NS}	151.53 ^{NS}	150.35			
K mmol/l	Aug	4.78	6.26	6.36	5.80 ^b	0.43 *	0.43 *	0.74 *
	Sep	5.06	6.90	9.30	7.09 ^a			
	Oct	5.76	5.30	5.88	5.65 ^b			
	Overall means	5.20 ^B	6.15 ^{AB}	7.18 ^A	6.18			
Ca mmol/l	Aug	5.55	7.36	13.44	8.79 ^{NS}	0.74 ^{NS}	0.73 *	1.28 *
	Sep	9.29	9.11	11.19	9.86 ^{NS}			
	Oct	8.34	8.90	9.83	9.02 ^{NS}			
	Overall means	7.73 ^B	8.46 ^B	11.49 ^A	9.22			
P mmol/l	Aug	2.08	2.89	6.16	3.71 ^{NS}	0.44 ^{NS}	0.44 *	0.77 *
	Sep	3.52	4.31	5.49	4.44 ^{NS}			
	Oct	3.26	3.22	3.83	3.44 ^{NS}			
	Overall means	2.295 ^B	3.78 ^B	5.16 ^A	3.86			

Means with different letters are statistically different ($P < 0.05$). A, B = Values with different letters on the same row differ at ($P < 0.05$), a, b = Values with different letters on the same column differ at ($P < 0.05$). NS: Non significant. * : Significant. Na: Sodium. K: Potassium. Ca: Calcium. P: Phosphors.

Table (12): Average values of some serum micro elements in pregnant Darshawy ewes fed on different forms of mineral additives.

Parameters	Month (M)	Treatments (Tr)			Overall means	± Standard Error		
		Control	Inorganic	Organic		M	Tr	Tr*M
Mg mg/l	Aug	3.65	4.07	4.58	4.10 ^{NS}	0.30 ^{NS}	0.30 ^{NS}	0.52 ^{NS}
	Sep	3.86	3.66	3.52	3.68 ^{NS}			
	Oct	4.08	2.67	3.30	3.35 ^{NS}			
	Overall means	3.86 ^{NS}	3.47 ^{NS}	3.80 ^{NS}	3.71			
Mn mg/l	Aug	0.07	0.03	0.02	0.04 ^{NS}	0.01 ^{NS}	0.01 ^{NS}	0.01 ^{NS}
	Sep	0.03	0.04	0.06	0.05 ^{NS}			
	Oct	0.04	0.04	0.05	0.04 ^{NS}			
	Overall means	0.05 ^{NS}	0.04 ^{NS}	0.05 ^{NS}	0.04			
Cu mg/l	Aug	2.01	2.00	2.36	2.12 ^{NS}	0.30 ^{NS}	0.30 ^{NS}	0.52 ^{NS}
	Sep	1.53	3.26	1.91	2.23 ^{NS}			
	Oct	1.47	1.35	1.75	1.52 ^{NS}			
	Overall means	1.67 ^{NS}	2.20 ^{NS}	2.01 ^{NS}	1.96			
Zn mg/l	Aug	6.35	5.96	7.15	6.49 ^{NS}	0.43 ^{NS}	0.43 [*]	0.74 [*]
	Sep	4.54	7.15	5.77	5.82 ^{NS}			
	Oct	4.20	7.90	5.67	5.92 ^{NS}			
	Overall means	5.03 ^B	7.00 ^A	6.20 ^{AB}	6.07			
Se mg/l	Aug	0.10	0.09	0.10	0.10 ^{NS}	0.01 ^{NS}	0.01 [*]	0.01 [*]
	Sep	0.07	0.11	0.09	0.09 ^{NS}			
	Oct	0.07	0.12	0.09	0.09 ^{NS}			
	Overall means	0.08 ^B	0.11 ^A	0.10 ^{AB}	0.09			
Fe µg/dl	Aug	44.62	46.41	50.59	47.21 ^{NS}	2.52 ^{NS}	2.47 ^{NS}	4.37 ^{NS}
	Sep	40.81	44.99	48.12	44.64 ^{NS}			
	Oct	50.59	48.23	52.70	50.51 ^{NS}			
	Overall means	45.34 ^{NS}	46.54 ^{NS}	50.47 ^{NS}	48.06			

Means with different letters are statistically different ($P < 0.05$). A, B = Values with different letters on the same row differ at ($P < 0.05$), NS: Non significant. *: Significant. Mg: Magnesium. Mn: Manganese. Cu: Copper. Zn: Zinc. Se: Selenium. Fe: Iron.

Table (13): Reproductive performance of Darshawy ewes as affected by different forms of mineral additives.

Item	Groups		
	Control	Inorganic	Organic
No. of ewes at start	15	15	15
No. of ewes died before mating	3	4	4
No. of ewes joined (ej)	12	11	11
No. of ewes conceived (ec)	11	11	11
Conception rate (ec/ej)	0.92	1.0	1.0
No. of ewes aborted	1	0	2
No. of ewes lambbed (el)	10	11	9
No. of lambs born (lb)	13	12	13
Average birth weight kg	2.55	2.96	2.85
Litter size at birth (lb/el)	1.30	1.09	1.44
Lambs died from birth to weaning	3	1	2
Mortality rate	0.23	0.08	0.15
No. of Lambs weaned	10	11	11
Average weaning weight kg	12.85	14.33	14.54
Litter size at weaning (lw/el)	1.00	1.00	1.22
Overall Reproductive efficiency (kg weaned / ewe joined)	10.71	14.33	14.54

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