

## CLINICAL STUDY ON THE IMPACT OF NUTRITIONAL DEFICIENCY ON THE HEALTH STATUS OF THE SHEEP IN NEW VALLEY GOVERNORATE

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### ABSTRACT

Sheep production is being encouraged in Egypt as a mean of improving the daily dietary protein intake for humans. Nutritional disorders are considered one of the serious distresses that influence the animal production. The current study aimed to investigate mineral deficiency in sheep and its effect on health status at different New Valley governorate cities. Clinical examination of the sheep was performed. Blood, water and soil samples were collected from different localities. Hematological parameters, serum total protein, albumin, globulin concentrations, kidney function tests and the activity of liver enzymes were estimated. Moreover, the values of some elements in soil and water were determined. Clinical examination showed various symptoms as weight reduction, lameness and eye and skin lesions. The biochemical results exhibited significant reduction of hemoglobin (Hb), mean corpuscular Hb, red blood cell count, hematocrit, lymphocytes, neutrophils, median cells and mean corpuscular Hb concentration. On the same hand, serum calcium, phosphorus ( $P^{3+}$ ), magnesium ( $Mg^{2+}$ ), copper ( $Cu^{2+}$ ), iron ( $Fe^{2+}$ ) and zinc ( $Zn^{2+}$ ) levels were reduced. Albumin concentration and albumin/globulin ratio were also diminished. There was also significant reduction of serum liver enzymes activity in Paris village. Analyzing of soil revealed decline in concentration of  $Mg^{2+}$  in all studied areas and  $Cu^{2+}$  and  $Zn^{2+}$  in Paris only. However, high values of  $P^{3+}$  and  $Fe^{2+}$  in all localities. Water samples analysis showed low concentrations of  $Cu^{2+}$ ,  $Zn^{2+}$  and manganese level. The present study confirmed that mineral deficiencies have great negative impacts on the healthy status of sheep in New Valley governorate.

**Key words:** Sheep; Clinical examination; Minerals; Nutritional deficiency.

### INTRODUCTION

Nutritional deficiency (ND) is a condition that leads to reduce the animal production and obstruct the trade and may

severe economic losses occur (Asin *et al.*, 2021). It may be occurred as a consequence of either insufficient diet, impaired absorption, and/or disorder in the metabolic process (Baugreet *et al.*, 2017).

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It was reported that macro and micro-minerals at optimum levels are important for animal growth and protection against some ND diseases. Calcium ( $Ca^{2+}$ ) is one of macro-element that important for muscle

contraction (Hu *et al.*, 2018), neurotransmission (Williams and Smith, 2018), bone growth and blood clotting (Wasilewski *et al.*, 2019). Phosphorus ( $P^{3+}$ ) is considered the second macro-mineral that crucial for cellular component as nucleic acid, phospholipids and high energy phosphate compounds (Vorland *et al.*, 2017). Additionally, magnesium ( $Mg^{2+}$ ) plays a critical role in activation of kinase and polymerase enzymes (Faraji *et al.*, 2021) and transportation of other cations such as  $Ca^{2+}$ , sodium (Na) and potassium across the cell membrane (Mathew and Panonnummal, 2021).

Regarding to micronutrients, it was found that copper ( $Cu^{2+}$ ) is essential for normal growth, hemoglobin (Hb) biosynthesis (Hill and Shannon, 2019) and activating some enzymes as superoxide dismutase, cytochrome C oxidase and monoamine oxidase (Espinosa and Stein, 2021). Further, iron ( $Fe^{2+}$ ) is another trace element in almost all organisms, as it occupies vital role in Hb synthesis, neurotransmission (Huang *et al.*, 2018), oxygen transport and DNA synthesis and repair (Ito *et al.*, 2021). On the same hand, the zinc ( $Zn^{2+}$ ) is one of essential trace element acts as an immunostimulant (Maares and Haase, 2016) and has multiple biological functions in growth and reproduction (Driessnack *et al.*, 2017).

Previous studies observed reduction of some macro minerals and microminerals in different cities in Egypt and other countries worldwide. Study of IBRAHIM *et al.* (2017) in Qena Governorate showed reduction of serum  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Fe^{2+}$ ,  $Cu^{2+}$ ,  $Zn^{2+}$  and  $P^{3+}$  by 58.82, 49.45, 30.09, 96.80, 60.94 and 92.31%, respectively, in sheep suffer from anemia, emaciation, loss of appetite, dehydration, diarrhea and pale mucous membrane. Further Morsy *et al.* (2020) study in Matrouh governorate noticed reduction of serum  $Cu^{2+}$ ,  $Zn^{2+}$  and  $Fe^{2+}$  percentage in sheep suffer from ND by 36.05, 68.57 and 88.37%, respectively. Moreover, ewes suffered from signs of ND

exhibited reduction in percentage  $Cu^{2+}$ ,  $Zn^{2+}$ ,  $Fe^{2+}$  and manganese ( $Mn^{2+}$ ) by 77.91, 40.07, 63.89 and 54.84% (EL-NASER *et al.*, 2013). On the same hand, sheep had liver trematodes which is one causes of ND exposed decrease in percentage of cobalt ( $Co^{2+}$ ),  $Mn^{2+}$ , selenium and  $Zn^{2+}$  by 51.02, 53.45, 58.21 and 37.15% (TUNCER *et al.*, 2020). Study of Saleh (2019) also proved reduction of serum  $Cu^{2+}$ ,  $Zn^{2+}$ ,  $Co^{2+}$ ,  $Mg^{2+}$  and  $Ca^{2+}$  in sheep had mineral deficiency in Iraq by 28.89, 83.70, 53.85, 35.58 and 67.13%, respectively.

The scope of current research is to investigate the ND health problem in sheep and its relation to surrounding environment as soil and water or related to other causes in New Valley governorate. This will be achieved through estimation of hematological and serum level of minerals in sheep suspected to be suffering ND syndrome.

## MATERIALS AND METHODS

### 1. Reagents, kits and solutions

Kits of  $Ca^{2+}$ ,  $P^{3+}$ ,  $Mg^{2+}$ ,  $Cu^{2+}$ ,  $Fe^{2+}$  and  $Zn^{2+}$ , total protein (TP), albumin, urea and creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were obtained from Spectrum Co. (Germany). Diethylenetriamine-pentacetic acid and ammonium acetate were obtained from Sigma Aldrich Co. (USA).

### 2. Experimental design

The study was conducted on 400 sheep, about 1-4 years of age (20-60 kg body weight). Sheep were selected from El-Dakhla (n=100), El-Kharga (n=150), and Paris (n=150) cities, New Valley Governorate. The experiment was conducted according to the ethical standards. The study was carried out from October 2020 till March 2021. At first, case history of sheep was recorded, then animal weighing and medical examination were performed. Following that and depending on obtained data from case history and

clinical examination, 30 animals were selected from each New Valley locality. These animals showed clinical signs of mineral deficiency including easily detached wool, anorexia, and loss of skin rigidity and elasticity, (mineral deficient group). Control healthy animals were selected from each city on the basis of case history, clinical examination (control group).

### 3. Clinical examination and blood, soil and water sampling

Clinical examination of sheep was conducted through examination of body temperature (BT), skin lesions, wool status, mucous membrane alterations, lameness conditions and eye lesions for 400 sheep distributed as following; a hundred animal (50 sheep from El-Kharga, and the 50 from Paris) was excluded. Blood samples were collected from jugular vein (n=100 from each city), and all efforts were done to minimize the distress. Blood samples were collected in two types of test tubes, one was with ethylene diamine tetra acetic acid (EDTA) that acts as an anticoagulant and the second was without anticoagulant and kept in an inclined position for 20 minutes at room temperature. Tubes with anticoagulant were kept without centrifugation and used for complete blood picture count (CBC), however the second tubes were centrifuged, and the clear serum was separated and stored at -20°C until analyzed. After CBC, sub-selection from these samples was done (n=30 from each locality) for biochemical analysis. Additionally, blood and serum samples were collected from control healthy sheep (n=30). On the other hand, top-soil samples were collected according to Association of Official Analytical Chemists (AOAC) recommendation (Lee, 1995). The collection of soil samples occurred on depth of 25-30 cm in each area that the animal was fed. Three soil samples were collected from each studied area and sealed in double thickness plastic bags and stored in cool dark condition until time of analysis.

Additionally, water samples were taken from the main source of water in El-Dakhla, El-Kharga and Paris (n=3 from each locality) and collected in sterile bottles and sent directly to the laboratory for the biochemical analysis (Hitchens, 1972).

### 4. Hematological parameters analysis

The hematological parameters were estimated in the Research Laboratory, Faculty of Veterinary Medicine, New Valley University, with Auto hematology Analyzer (KT-60, China). These parameters included red blood cell count (RBC), the concentration of Hb, hematocrit (HCT), lymphocytes (LYM), neutrophils (NEU) and median cells (MID) percentage, mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), white blood cell count (WBC) and platelets (PLT).

### 5. Serum biochemical parameters analysis

All estimated biochemical parameters were carried out using Biochemistry Analyzer (Prietest COMPACT, India) in the Research Laboratory, Faculty of Veterinary Medicine, New Valley University. Serum  $\text{Ca}^{2+}$ ,  $\text{P}^{3+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{2+}$  and  $\text{Zn}^{2+}$  concentrations were estimated photometrically according to instructions of kits manufactures. Furthermore, serum total protein (TP), albumin, urea and creatinine concentrations were analyzed. Both globulin concentration and albumin/globulin (A/g) ratio were calculated. The activity of liver enzymes, ALT and AST were also determined by kinetic commercial kits and expressed as U/L.

### 6. Soil analysis

The mineral concentration in the soil was analyzed according to Whitehead (1979). In brief, the collected samples were dried at 40°C in the oven (DFZ LTD, China), passed through nylon 2 mm opening sieve and finally grounded in an agate ball mill. The prepared soil was subjected to one of the following treatments either treated by i: diethylenetriamine-pentacetic acid to

estimate the levels of  $\text{Fe}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$ , or ii: ammonium acetate to analyze  $\text{Ca}^{2+}$  and  $\text{P}^{3+}$  concentration. The results were compared to reference values of Regional Soil Fertility Coefficients (1999). Soil analysis was done in Regional Laboratory for Water and Soil Analysis at the Directorate of Agriculture in New Valley.

### 7. Water analysis

The water samples were analyzed for  $\text{Fe}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{P}^{3+}$  by using computerized atomic absorption technique (GBC-932-AA) air-acetylene type according to manufacture instructions and done by colorimetric titration methods (Laglera and Monticelli, 2017; Özzeybek *et al.*, 2017). The obtained values were expressed in mg/L. The results were compared to critical level of international standard for drinking water (WHO, 1971). Water analysis technique was performed in Biological Laboratory for Food Analysis, El-Kharga Specialized Hospital.

### 8. Statistical analysis

The obtained data were statistically analyzed using SPSS (Version 25), both descriptive (Mean  $\pm$  S.E.) and tests of significance by One way ANOVA (Using Duncan's followed by POST HOC test) were performed. The results were presented as Mean  $\pm$  SE and the significant P value was set to  $< 0.05$ .

## RESULTS

### 1. Clinical examination results

The data obtained from case history revealed that animals suffered from loss of appetite in percentage 44.0, 16.7 and 23.3 in El-Dakhla, El-Kharga and Paris, respectively. This case history was confirmed by decline in body weight (Bwt) approximately 42.0% in El-Dakhla, 12.7% in El-Kharga and 15.3% in Paris from the total inspected sheep number (n=100) in each locality. Further it was observed presence of Bwt reduction by 62.5, 46.0

and 63.7% in El-Dakhla, El-Kharga and Paris, respectively when compared to control (Table 1).

Additionally, the clinical examination of sheep exhibited presence of 10.0% in El-Dakhla and 9.3% in Paris had a higher BT however in El-Kharga all examined sheep were normal. By comparing the BT of sheep in El-Dakhla, El-Kharga and Paris by control, noted that there wasn't any significant difference ( $p \leq 0.05$ ) (Table 1).

Furthermore, it was observed presence of skin lesions as alopecia, scarp, dark spots, dermatitis and papilloma by 45.0, 3.3 and 11.3% in El-Dakhla, El-Kharga and Paris, respectively. Concerning to the wool status, mucous membrane and lameness, it was noted presence of multiple alterations respectively as follow, 35.0%, 25.0% and 11.0% in El-Dakhla, 21.3%, 6.0% and 14.0% in El-Kharga and 10.0%, 14.7% and 5.3% in Paris from the total inspected sheep (n=100) during our study. Further, it was observed in El-Dakhla and Paris 11.0% and 14.0% of sheep respectively were suffered from eye lesions as conjunctivitis and pink eye and there was enlargement of lymph node in 9.0% and 4.7% of them, while those two signs were not noticed in El-Kharga. Moreover, via observing the nervous manifestation like depression, disorientation, flaccid lip and severe salivation, it was found only 4.0% in El-Dakhla had the signs.

Finally, from all previous clinical examination, we concluded that there was 50.0%, 25.0% and 30.0% from the examined sheep in El-Dakhla, El-Kharga, and Paris localities (n=100 in each locality), respectively, had a clinical mineral deficiency symptom (Figs.1 and Figs.2).

### 2. Hematological analyses in sheep from different localities in New Valley

The current data exhibited significant reduction of Hb concentration by 23.4 and 18.4% in collected samples in El-Dakhla and Paris, respectively compared to control

group. Additionally, there was significant decline by 10.8% in El-Dakhla and 4.9% in Paris when compared to El-Kharga. Further, it was noticed only significant difference in MCH concentration in El-Dakhla comparing to control, El-Kharga and Paris, by 13.7, 6.25 and 3.8-percentage decrease. RBC showed significant decline in samples collected from El-Dakhla by 11.5, 4.7 and 7.1% compared to control, El-Kharga and Paris, respectively. Significant decrease was also observed in PLT in Paris in comparison to control, El-Dakhla and El-Kharga approximately 31.2, 32.6 and 23.0%. Additionally, HCT and NEU were dropped 16.7 and 60.0% while LYM was increased by 38.4% in comparison to control and also, HCT and NEU reduced to 0.07 and 0.72-folds and LYM was increased by 0.7-fold in comparison to El-Kharga and further HCT and NEU were reduced by 10.2% and 1.3-fold comparing to Paris, however LYM was increased by 31.5%. Concerning to MID, it was only declined by 31.2% in El-Dakhla, however elevated by 83.1 and 66.2% in El-Kharga and Paris, respectively. Additionally, MCHC revealed reduction by 7.8% compared to control and 6.25 % compared to El-Kharga. Finally, when compared MCHC by RBC in control, El-Dakhla, El-Kharga and Paris it was noted an increase level by 76.9, 77.9, 77.5 and 76.1%, respectively. Hematological data was presented in Table (2).

### 3. Serum minerals concentration in sheep from different localities in New Valley

Serum data of mineral analysis in Table (3) revealed that reduction of serum  $\text{Ca}^{2+}$ ,  $\text{P}^{3+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{2+}$  and  $\text{Zn}^{2+}$  by 14.0, 28.6, 67.2, 30.4, 44.1 and 16.2% in El-Dakhla in mineral deficient group, respectively, in comparing to control one. On the other hand, it was reported that  $\text{Ca}^{2+}$  concentration was elevated by 7.6 and 8.7% in El-Kharga when compared to El-Dakhla and Paris. Also, in Paris  $\text{P}^{3+}$ ,  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$  levels were increased by 21.4, 8.2 and 3.7%

compared to El-Dakhla and by 23.1, 11.4 and 6.1% compared to El-Kharga.

### 4. Serum TP, albumin and globulin concentration and A/G ratio in sheep from different localities in New Valley

The obtained results in Table (4) showed that there was decrease in TP, albumin and A/G ratio in mineral-deficient group compared by control ones, however this reduction was significant in some studied localities and non-significant in others. In El-Kharga, only TP level was significantly decreased by 17.6% compared to control one and by 10.9 and 15.2% in comparison to El-Dakhla and Paris. On the same hand, albumin and A/G ratio were declined by 26.7 and 33.8% in El-Dakhla, 41.2 and 46.0% in El-Kharga and 26.7 and 38.4% in Paris when compared to control one. Further, globulin concentration in Paris only exhibited elevation by 21.7, 7.6 and 13.0% in comparison to control, El-Dakhla and El-Kharga, respectively.

### 5. Serum urea and creatinine concentration and ALT and AST activity in sheep from different localities in New Valley

The activity of ALT and AST was determined. It was noted that ALT activity was increased by 39.8% in Paris, however there was not significant variation in El-Dakhla and El-Kharga when compared to control one. Also, ALT was double elevated in Paris by 50.9 and 53.9%, comparing to El-Dakhla and El-Kharga. Moreover, AST activity was only significant raised in Paris by 41.7, 37.5 and 41.1% compared to control, El-Dakhla and El-Kharga (Table 5).

Serum urea was not significant in studied areas compared to control, while serum creatinine level exhibited significant reduction in El-Kharga by 23.1, 25.1 and 27.9% comparing to control, El-Dakhla and Paris (Table 5).



**Figs.1:** Sheep about 3.5-4 years of age with highly detached wool, alopecia and emaciation.



**Figs.2:** Sheep about 3.5-4 years of age suffer from lameness and emaciation.

**Table 1:** Mean values ( $\pm$  SE) of Bwt and BT in healthy control and mineral-deficient sheep from different localities in New Valley governorate

	<b>Control (n= 100)</b>	<b>El-Dakhla (n= 100)</b>	<b>El-Kharga (n= 100)</b>	<b>Paris (n= 100)</b>
Bwt (kg)	48.11 $\pm$ 1.53 <sup>a</sup>	18.05 $\pm$ 1.17 <sup>b</sup>	25.98 $\pm$ 0.38 <sup>b</sup>	17.45 $\pm$ 0.29 <sup>b</sup>
BT ( $^{\circ}$ C)	39.04 $\pm$ 0.10 <sup>a</sup>	39.74 $\pm$ 0.19 <sup>a</sup>	39.20 $\pm$ 0.03 <sup>a</sup>	39.18 $\pm$ 0.09 <sup>a</sup>

Values represent mean  $\pm$  SE. Means with different superscript at the same row are significantly different at  $p \leq 0.05$ .

**Table 2:** Mean values ( $\pm$  SE) of hematological parameters in healthy control and mineral-deficient sheep from different localities in New Valley governorate

	<b>Control (n= 100)</b>	<b>El-Dakhla (n= 100)</b>	<b>El-Kharga (n= 100)</b>	<b>Paris (n= 100)</b>
RBC ( $10^6$ /ul)	8.38 $\pm$ 0.08 <sup>a</sup>	7.42 $\pm$ 0.22 <sup>b</sup>	7.77 $\pm$ 0.20 <sup>a</sup>	7.98 $\pm$ 0.10 <sup>a</sup>
Hb (g/dl)	9.69 $\pm$ 0.14 <sup>a</sup>	7.42 $\pm$ 0.23 <sup>b</sup>	8.32 $\pm$ 0.23 <sup>a</sup>	7.91 $\pm$ 0.17 <sup>b</sup>
HCT (%)	26.93 $\pm$ 0.44 <sup>a</sup>	22.44 $\pm$ 0.58 <sup>b</sup>	24.10 $\pm$ 0.65 <sup>a</sup>	24.98 $\pm$ 0.50 <sup>a</sup>
MCV (fL)	32.16 $\pm$ 0.48 <sup>a</sup>	30.46 $\pm$ 0.69 <sup>a</sup>	31.17 $\pm$ 1.20 <sup>a</sup>	31.34 $\pm$ 0.50 <sup>a</sup>
MCH (pg)	11.64 $\pm$ 0.22 <sup>a</sup>	10.05 $\pm$ 0.28 <sup>b</sup>	10.72 $\pm$ 0.25 <sup>a</sup>	10.45 $\pm$ 0.35 <sup>a</sup>
MCHC (g/dl)	36.33 $\pm$ 0.75 <sup>a</sup>	33.51 $\pm$ 0.64 <sup>b</sup>	34.53 $\pm$ 0.84 <sup>a</sup>	33.35 $\pm$ 0.87 <sup>b</sup>
WBC ( $10^3$ /ul)	10.78 $\pm$ 0.74 <sup>a</sup>	12.33 $\pm$ 0.96 <sup>a</sup>	9.13 $\pm$ 1.19 <sup>a</sup>	10.23 $\pm$ 0.42 <sup>a</sup>
LYM ( $10^3$ /ul)	7.91 $\pm$ 0.72 <sup>a</sup>	10.95 $\pm$ 0.92 <sup>b</sup>	6.43 $\pm$ 0.35 <sup>a</sup>	7.50 $\pm$ 0.52 <sup>a</sup>
NEU ( $10^3$ /ul)	2.10 $\pm$ 0.30 <sup>a</sup>	0.84 $\pm$ 0.20 <sup>b</sup>	3.07 $\pm$ 0.49 <sup>a</sup>	1.96 $\pm$ 0.30 <sup>a</sup>
MID ( $10^3$ /ul)	0.77 $\pm$ 0.22 <sup>a</sup>	0.53 $\pm$ 0.11 <sup>b</sup>	1.41 $\pm$ 0.09 <sup>c</sup>	1.28 $\pm$ 0.20 <sup>c</sup>
PLT (k/ul)	678.2 $\pm$ 52.83 <sup>a</sup>	692.2 $\pm$ 48.48 <sup>a</sup>	605.2 $\pm$ 68.18 <sup>a</sup>	466.3 $\pm$ 30.77 <sup>b</sup>

Values represent mean  $\pm$  SE. Means with different superscript at the same row are significantly different at  $p \leq 0.05$ .

**Table 3:** Mean values ( $\pm$  SE) of serum minerals concentrations in healthy control and mineral-deficient sheep from different localities in New Valley governorate

	<b>Control (n= 30)</b>	<b>El-Dakhla (n= 30)</b>	<b>El-Kharga (n= 30)</b>	<b>Paris (n= 30)</b>
Ca <sup>2+</sup> (mg/dl)	8.93 $\pm$ 0.12 <sup>a</sup>	7.68 $\pm$ 0.23 <sup>b</sup>	8.26 $\pm$ 0.12 <sup>a</sup>	7.60 $\pm$ 0.23 <sup>b</sup>
P <sup>3+</sup> (mg/dl)	4.12 $\pm$ 0.27 <sup>a</sup>	2.94 $\pm$ 0.18 <sup>b</sup>	2.90 $\pm$ 0.21 <sup>b</sup>	3.57 $\pm$ 0.25 <sup>a</sup>
Mg <sup>2+</sup> (mg/dl)	0.64 $\pm$ 0.07 <sup>a</sup>	0.21 $\pm$ 0.03 <sup>b</sup>	0.28 $\pm$ 0.07 <sup>b</sup>	0.27 $\pm$ 0.05 <sup>b</sup>
Cu <sup>2+</sup> ( $\mu$ g/dl)	110.30 $\pm$ 6.34 <sup>a</sup>	76.79 $\pm$ 5.66 <sup>b</sup>	74.59 $\pm$ 2.82 <sup>b</sup>	83.12 $\pm$ 6.86 <sup>c</sup>
Fe <sup>2+</sup> ( $\mu$ g/dl)	109.60 $\pm$ 11.57 <sup>a</sup>	61.24 $\pm$ 8.75 <sup>b</sup>	55.61 $\pm$ 1.08 <sup>b</sup>	58.49 $\pm$ 5.15 <sup>b</sup>
Zn <sup>2+</sup> ( $\mu$ g/dl)	98.81 $\pm$ 2.72 <sup>a</sup>	82.85 $\pm$ 3.20 <sup>b</sup>	80.96 $\pm$ 2.71 <sup>b</sup>	85.88 $\pm$ 2.76 <sup>a</sup>

Values represent mean  $\pm$  SE. Means with different superscript at the same row are significantly different at  $p \leq 0.05$ .

**Table 4:** Mean values ( $\pm$  SE) of serum TP, albumin and globulin concentration and A/G ratio in healthy control and mineral-deficient sheep from different localities in New Valley governorate

	Control (n= 30)	El-Dakhla (n= 30)	El-Kharga (n= 30)	Paris (n= 30)
TP (g/dl)	7.26 $\pm$ 0.21 <sup>a</sup>	6.71 $\pm$ 0.11 <sup>a</sup>	5.98 $\pm$ 0.08 <sup>b</sup>	7.05 $\pm$ 0.15 <sup>a</sup>
Albumin (g/dl)	3.74 $\pm$ 0.12 <sup>a</sup>	2.74 $\pm$ 0.09 <sup>b</sup>	2.20 $\pm$ 0.13 <sup>b</sup>	2.78 $\pm$ 0.10 <sup>b</sup>
Globulin (g/dl)	3.51 $\pm$ 0.17 <sup>a</sup>	3.97 $\pm$ 0.10 <sup>a</sup>	3.78 $\pm$ 0.14 <sup>a</sup>	4.27 $\pm$ 0.09 <sup>b</sup>
A/G ratio	1.05 $\pm$ 0.77 <sup>a</sup>	0.70 $\pm$ 0.04 <sup>b</sup>	0.57 $\pm$ 0.04 <sup>b</sup>	0.65 $\pm$ 0.02 <sup>b</sup>

Values represent mean  $\pm$  SE. Means with different superscript at the same row are significantly different at  $p \leq 0.05$ .

**Table 5:** Mean values ( $\pm$  SE) of serum liver enzymes activity and kidney function tests in healthy control and mineral-deficient sheep from different localities in New Valley governorate

	Control (n= 30)	El-Dakhla (n= 30)	El-Kharga (n= 30)	Paris (n= 30)
ALT (U/L)	21.16 $\pm$ 1.12 <sup>a</sup>	19.60 $\pm$ 0.84 <sup>a</sup>	19.22 $\pm$ 1.13 <sup>a</sup>	29.58 $\pm$ 1.48 <sup>b</sup>
AST (U/L)	26.38 $\pm$ 1.32 <sup>a</sup>	27.17 $\pm$ 0.82 <sup>a</sup>	26.48 $\pm$ 1.75 <sup>a</sup>	37.37 $\pm$ 1.48 <sup>b</sup>
Urea (mg/dl)	46.09 $\pm$ 2.05 <sup>a</sup>	53.00 $\pm$ 4.47 <sup>a</sup>	39.50 $\pm$ 1.89 <sup>a</sup>	53.48 $\pm$ 3.16 <sup>a</sup>
Creatinine (mg/dl)	1.09 $\pm$ 0.06 <sup>a</sup>	1.12 $\pm$ 0.06 <sup>a</sup>	0.84 $\pm$ 0.03 <sup>b</sup>	1.16 $\pm$ 0.04 <sup>a</sup>

Values represent mean  $\pm$  SE. Means with different superscript at the same row are significantly different at  $p \leq 0.05$ .

**Table 6:** Extractable mineral concentrations in soil from different localities in New Valley governorate

	Ca <sup>2+</sup> (mEq/100 g)	P <sup>3+</sup> (ppm)	Mg <sup>2+</sup> (ppm)	Cu <sup>2+</sup> (ppm)	Fe <sup>2+</sup> (ppm)	Zn <sup>2+</sup> (ppm)	
El-Dakhla (n= 3)	4.2 <sup>N</sup>	30.6 <sup>H</sup>	4.92 <sup>L</sup>	4.58 <sup>H</sup>	78.0 <sup>H</sup>	4.7 <sup>H</sup>	
El-Kharga (n= 3)	4.9 <sup>N</sup>	32.2 <sup>H</sup>	5.66 <sup>L</sup>	3.2 <sup>H</sup>	21.0 <sup>H</sup>	2.08 <sup>H</sup>	
Paris (n= 3)	2.8 <sup>N</sup>	21.3 <sup>H</sup>	2.0 <sup>L</sup>	0.4 <sup>L</sup>	10.52 <sup>H</sup>	0.88 <sup>L</sup>	
Reference values	L	< 2.4	< 10.0	< 50	< 0.5	< 4.0	< 1.0
	N	2.4-5	11-15	50-70	0.5-1.0	4.0-6.0	1.0-2.0
	H	> 5	> 15	> 70	> 1.0	> 6.0	> 5.0

Values with superscripts (L) is lower than reference values of Regional Soil Fertility Coefficients, (N) is the normal reference values of Regional Soil Fertility Coefficients and (H) is higher than reference values of Regional Soil Fertility Coefficients.

**Table 7:** Mineral concentrations in water from different localities in New Valley governorate

	<b>Ca<sup>2+</sup> carbonate (mg/L)</b>	<b>Fe<sup>2+</sup> (mg/L)</b>	<b>Cu<sup>2+</sup> (mg/L)</b>	<b>Zn<sup>2+</sup> (mg/L)</b>	<b>Mn<sup>2+</sup> (mg/L)</b>
El-Dakhla (n= 3)	12 <sup>L</sup>	0.5 <sup>N</sup>	< 0.1 <sup>L</sup>	< 0.1 <sup>L</sup>	< 0.1 <sup>L</sup>
El-Kharga (n= 3)	16 <sup>L</sup>	0.4 <sup>N</sup>	< 0.1 <sup>L</sup>	< 0.1 <sup>L</sup>	< 0.1 <sup>L</sup>
Paris (n= 3)	32 <sup>L</sup>	0.3 <sup>N</sup>	< 0.1 <sup>L</sup>	< 0.1 <sup>L</sup>	< 0.1 <sup>L</sup>
Critical levels	500	0.3	2.0	3.0	0.4

Values with superscripts (L) is lower than the critical levels of international standard of drinking water (WHO, 1971), respectively and (N) is the normal critical level of international standard of drinking water (WHO, 1971).

## DISCUSSION

Nutrients are crucial for the general body health. They offer the needed raw materials for different tissues. Among these nutrients, the trace elements which have an important role in various biochemical reactions, as they act as cofactors for many enzymes (Nwosu, 2019). Both macro and micro-nutrients obtained from diet, minerals and vitamins are important micro-nutrients that required in trace amount stimulating necessary different biological reaction for cell metabolism in livestock (Lee *et al.*, 2002). Minerals contents and balance are important for productive and reproductive life of small ruminant (Vázquez-Armijo *et al.*, 2011). Minerals perform chief biological and physiological role (Nwosu, 2019). Undesirable effect and health problems may result from decreased mineral feed concentrations, absorption and bioavailability (Arshad *et al.*, 2021).

The present study disclosed that animals under investigation acquired significant mineral deficiency. This greatly affects health status of examined animals. Our results showed a significant reduction of serum Ca<sup>2+</sup>, P<sup>3+</sup>, Mg<sup>2+</sup>, Cu<sup>2+</sup>, Fe<sup>2+</sup> and Zn<sup>2+</sup> concentrations ( $p \leq 0.05$ ) in El-Dakhla locality. Those minerals concentrations except Ca<sup>2+</sup> were also less than control in El-Kharga locality. However, in Paris locality P<sup>3+</sup> and Zn<sup>2+</sup> concentrations were not significant.

Regarding to serum Zn<sup>2+</sup> concentration, the current study revealed a decrease in serum Zn<sup>2+</sup> concentration, led to physiological defects accompanied with wide range of disorders, including anorexia, weight loss, growth retardation, skin lesions, swelling of the eye lid, alopecia and dermatitis, this finding matches with the results obtained by Sloup *et al.* (2017). Concurrently joint stiffness and reduction in the hematological parameters were recorded. This finding agreed with the observation of Song and Shen (2020), who attributed this disturbance to the decline in some Zn<sup>2+</sup> dependent metabolic enzymes as alkaline phosphatase and lactic acid dehydrogenase. Fertility wise, our study clinically revealed marked reduction in both male and female fertility these findings support the outcome presented by Najafzadeh *et al.* (2013) who stated that there is marked fertility reduction in ruminant suffering Zn<sup>2+</sup> deficiency (Tables 2 and 3).

The current study revealed reduction of serum Cu<sup>2+</sup> in all studied areas. This led to anemia, poor growth, wool keratinization and bone disorders of sheep. These findings agreed with Mandour *et al.* (2021). Cu<sup>2+</sup> deficiency reduce Fe<sup>2+</sup> absorption from the gut as Cu<sup>2+</sup> can modulate the hephaestin-a multi- Cu<sup>2+</sup> oxidase required for optimal Fe<sup>2+</sup> absorption (Matak *et al.*, 2013). This fact justifies the reduced appetite, emaciation, unsteady gait of sheep suffering from serum Cu<sup>2+</sup> decrease. This finding goes with the postulate of Wu *et al.* (2020) who assured the decreased performance,

appetite, anemia and economic losses due to hypocupermia. The deficiency of  $\text{Cu}^{2+}$  may be attributed to high concentration of other elements (Thorndyke *et al.*, 2021) as molybdenum (MO) and sulfur (S) in the soil which lead to formation of  $\text{MO-Cu}^{2+}$  or  $\text{S-Cu}^{2+}$  complex which are poorly absorbed via the intestine. Moreover, some authors (Abramowicz *et al.*, 2019; Shen and Song 2021) attributed serum  $\text{Cu}^{2+}$  decline to its decrease in the soil and forage. This finding assures this study notice in Paris locality. In this respect, some authors (Wu *et al.*, 2020) stated that, hypocupermia not only depends on the total  $\text{Cu}^{2+}$  contents but also it is greatly dependent on other factors affecting absorption and utilization so,  $\text{Cu}^{2+}$  deficiency has negative impact (Table 3).

$\text{Ca}^{2+}$  and  $\text{P}^{3+}$ , are two major component minerals of the ruminant body. They play a major role in neuro transmission and other metabolic activities, besides  $\text{P}^{3+}$  is key element in maintaining acid-base balance, and consider vital element in energy skeleton within the animal body (Yano *et al.*, 1991). It also important for ruminal microbial fermentation and protein synthesis and the insufficient dietary  $\text{P}^{3+}$  affect digestibility (Puggaard *et al.*, 2011). Hypocalcemia and hypophosphatemia are partially common in ruminant (Goff, 2000).

Prolonged dry period with dead pasture may cause low  $\text{P}^{3+}$  level. This results decreased the growth and production and caused bone softness, lameness and reduced the appetite (Masters and White, 1996). This finding agreed with our result which showed decreased serum concentration of  $\text{P}^{3+}$  level. Furthermore osteoporosis and decrease bone density, increased fracture risk accompany marked  $\text{Ca}^{2+}$  deficiency (Theobald, 2005). Common symptoms of reduced  $\text{Ca}^{2+}$  level are reduced growth rate and milk production, enlarged joints, tremors and general inertia (Masters and White, 1996) (Table 3).

The current study showed decrease of serum  $\text{Fe}^{2+}$  concentration that causes

decline in the most hematological parameters. This come in accordance with Yatoo *et al.* (2013), who stated that  $\text{Fe}^{2+}$  deficiency influences the performance and production of sheep. Several reasons of  $\text{Fe}^{2+}$  deficiency, though its higher level in soil and normal in drinking water as affection of the sheep with infectious agent (Kojouri and Shirazi, 2007), and fascioliasis (Kojouri *et al.*, 2013). This may be a defensive mechanism made by the body, in which the  $\text{Fe}^{2+}$  is sequestered from circulation into storage form primarily in liver and bone marrow in trail to make the  $\text{Fe}^{2+}$  unavailable to the pathogens to limit its activities and growth, however this negatively affect the process of erythropoiesis (Carlson, 1996) (Tables 2, 3, 6 and 7).

Additionally, it was observed that several factors can affect  $\text{Mg}^{2+}$  absorption and utilization in ruminant, like parathyroid hormone that decreasing its urinary excretion and stimulating bone resorption, thus releasing  $\text{Mg}^{2+}$  into the extracellular fluid,  $\text{Mg}^{2+}$  urinary excretion and absorption from the gastrointestinal tract which may be due to high dietary  $\text{K}^+$  that decrease the  $\text{Mg}^{2+}$  absorption (Hardwick *et al.*, 1991). The reported hypomagnesemia in our study was chiefly due to low intake of  $\text{Mg}^{2+}$  in food. This was confirmed by presence of low  $\text{Mg}^{2+}$  level in soil. This finding matches results obtained by Kumssa *et al.* (2019) and higher  $\text{Ca}^{2+}$  and  $\text{Na}$  intake. This agreed with Pickering *et al.* (2020) (Tables 3 and 6).

Proper digestion in rumen is important key factor for animal general health, proper protein fermentation which is the key factor for liver albumin and globulin production. It was noted the importance of minerals in normal rumen function. These co-enzymes possess potential role in binding pathogenic microorganisms and toxic compounds (Saleem *et al.*, 2012), possibly, due to their high adsorption capacity (Slamova *et al.*, 2011) (Table 4). Hepatocyte integrity can be monitored via AST and ALT levels

(Bobe *et al.*, 2004), where lower minerals concentration negatively affect ruminal micro-flora, this may lead to production of toxic products (Wang *et al.*, 2013). Increasing liver enzymes either direct through increased liver detoxification due to toxic load or indirect because of increase oxidative stress (Guo *et al.*, 2017) (Table 5). Similarly Slamova *et al.* (2011) attributed the beneficial role of mineral on liver due to its high capability of toxic adsorption, this facts agree with this study results that showed decreased liver activity and lower albumin, globulin as well as increased in liver enzymes.

## CONCLUSIONS

Current study showed that, naturally grazed sheep in different New Valley localities, exhibit variable degrees of serum minerals deficiency that led to marked decreases in performance, blood parameters. Therefore, regulatory examination and monitoring health status are recommended to increase the economical profit and production of these animals.

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## CONFLICT OF INTEREST DISCLOSURE

The authors declare no conflicts of interest associated with this manuscript.

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## دراسة إكلينيكية علي تأثير النقص الغذائي علي الحالة الصحية في الأغنام في محافظة الوادي الجديد

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تعد تربية الأغنام في مصر إحدى الوسائل المستخدمة لتحسين البروتين الغذائي اليومي للإنسان لذا تعتبر الاضطرابات التغذوية من الأمراض التي تمثل خطورة عالية على الإنتاج الحيواني. وقد هدفت الدراسة الحالية إلى إجراء دراسة إكلينيكية لنقص المعادن في الأغنام في بعض المناطق بمحافظة الوادي الجديد مثل الداخلة ، الخارجة ، وباريس. وقد تمت إجراء الدراسة الإكلينيكية للأغنام وبعد ذلك تم جمع عينات الدم والماء والترية من مختلف مناطق الدراسة وتم تقدير مقاييس الدم وتقدير تركيزات البروتين والألبومين والجلوبولين وفحوصات وظائف الكلى. وأيضاً تم تحليل نشاط إنزيمات الكبد. كما تم تحديد قيم بعض العناصر في التربة والمياه. وقد أظهرت نتائج الدراسة الإكلينيكية وجود أعراض مختلفة مثل ضعف الشهية ونقص الوزن والعرج وأفات العين والجلد في بعض الأغنام. وأظهرت أيضاً النتائج البيوكيميائية انخفاضاً كبيراً في الهيموجلوبين ، ومتوسط خضاب الدم في الجسم ، وعدد خلايا الدم الحمراء ، والهيماتوكريت ، والخلايا الليمفاوية ، والعدلات ، والخلايا المتوسطة ، ومتوسط تركيز خضاب الدم في الجسم. وقد لوحظ أيضاً تقليل معدل الكالسيوم والفوسفور والمغنيسيوم والنحاس والحديد والزنك. كما انخفض تركيز الألبومين ونسبة الألبومين / الجلوبيولين. وقد كان هناك أيضاً انخفاض كبير في نشاط إنزيمات الكبد في الدم في منطقة باريس فقط. وقد أوضحت النتائج أيضاً انخفاض ملحوظ في تركيز المغنيسيوم في جميع المناطق بينما في منطقة باريس تم إنخفاض عنصر النحاس والزنك فقط بينما كان هناك ارتفاع لبعض العناصر في جميع المراكز مثل عنصر الفوسفور والحديد. بينما أظهر تحليل عينات المياه تراكيز منخفضة من عناصر النحاس ، الزنك والمنجنيز. وقد توصل البحث الحالي إلى أن نقص المعادن من الأسباب المؤثرة على أداء الأغنام وإنتاجها وتكاثرها في محافظة الوادي الجديد.