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CLINICAL AND LABORATORY EVALUATION ON NUTRITIONAL DEFICIENCY IN SHEEP IN EL-MINIA GOVERNORATE, EGYPT

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ABSTRACT

This study aimed to detect changes in haemato-biochemical constituents, selected minerals, vitamin A, and total antioxidant capacity (TAC) in sheep that showed signs of nutritional deficiency and identify the associated risk factors. Our study involved 150 sheep of various ages and sexes (lambs under 1 year old and adults of both sexes male and female aged 2 ± 0.5 years old) at Abo-korkas center in El-Minia governorate, Egypt. All sheep underwent clinical and laboratory examinations. The animals were divided into two groups: a diseased group of 90 sheep exhibiting signs such as alopecia, easily detached wool, and pica, and a control group of 60 healthy sheep. Blood, soil, and water samples were collected from August 2022 to September 2023. Results of different age and sex groups indicated significant reductions in hemoglobin (Hb), red blood cell count (RBC), zinc (Zn^{2+}), copper (Cu^{2+}), calcium (Ca^{2+}), vitamin A, TAC, and total protein content in the blood of diseased group compared to control. Soil analysis showed decreased Zn^{2+}, Cu^{2+}, and Ca^{2+} concentrations, along with a high concentration of Fe^{2+}, while water samples revealed low levels of Zn^{2+}, Cu^{2+}, Fe^{2+}, and Ca^{2+}.The findings suggest a significant relationship between nutritional deficiency in sheep and alterations in haemato-biochemical constituents, mineral status, vitamin A, and total antioxidant capacity, influenced by factors such as sex, age, health status, and mineral content in soil and water.

Keywords: Nutritional deficiency; Total antioxidant capacity; Minerals; Vitamin A; Soil

INTRODUCTION

Nutritional deficiency (ND) diseases are conditions that reduce animal production, obstructs trade, and may severe economic losses occur (Asin *et al.,* 2021).

Deficiencies in minerals, whether primary or secondary pose major obstacles in livestock production due to their links to reduced production, reproductive problems, and various health issues (Riaz & Muhammad, 2018). Furthermore, vitamin deficiencies exhibit serious health problem (Lee & Dabrowski, 2004).

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It can be caused by an inadequate diet, problems with absorption, and/or disruptions in the metabolic process (Baugreet *et al.,* 2017), soil or plants have insufficient mineral content, when certain elements hinder the plant's ability to utilize others, or certain elements conflict with one another (Hefnawy & Tórtora-Pérez, 2010).

The deficiency of a single mineral is seldom observed under field conditions, whereas deficiencies affecting multiple minerals are more commonly encountered (Salem, 2017). Macro and micro minerals have been identified as essential nutrients (Dar *et al.,* 2014).

Micro elements like copper, zinc, iron, and selenium are required for normal biochemical processes in the body, being a part of several enzymes (Evans & Halliwell, 2001), and essential components of certain endogenous antioxidants (Heidarpour *et al.,* 2013).

Regarding to macro minerals, calcium and phosphorous are essential minerals for kidney and skeletal development and function, in addition they are regulating various physiological processes such as cellular metabolism, proliferation, and protein synthesis (Stenhouse *et al.,* 2022).

Minerals requirements, along with deficiency and toxicity thresholds, vary according to factors such as age, sex, production level, activity, species, and genetic strain of the animal. Age, specifically, influences mineral needs, as younger animals, especially pre-ruminants, typically exhibit higher mineral absorption efficiency compared to older animals (Khan *et al.,* 2021; Schweinzer *et al.,* 2017).

Moreover, the susceptibility of animals to minerals imbalances can be affected by gender, likely due to differences in growth rates and physiological functions. Furthermore, environmental stresses, such as exposure to heat or cold, have a notable effect on serum biochemical parameters in animals (Dar *et al.,* 2014).

Antioxidants in animal production and health are primarily vitamins and minerals, including vitamin A (beta-carotene), vitamin C, vitamin E, Selenium, copper, and zinc (Sorci & Faivre, 2009; Weiss *et al.,* 2004). Antioxidants prevent cell deterioration by lowering free radicals, which are reactive chemical products that can damage macromolecules such as lipids, carbohydrates, proteins, and nucleic acids (Aktas *et al.,* 2017; Cinar *et al.,* 2018; Masters, 2018).

The stress caused by trace element deficiencies and hot climatic conditions generally increases the production of free radicals, resulting in oxidative stress (Saleh *et al.,* 2008).

Oxidative stress represents an active area of research within veterinary medicine and has been associated with multiple diseases (Lykkesfeldt & Svendsen, 2007).

Oxidative stress refers to an imbalance where oxidants outweigh the antioxidant system. This stress contributes to cellular and molecular tissue damage across various diseases (Ercan & Fidanci, 2012).

Additionally, oxidative stress poses significant risks as it often lacks clinical symptoms and requires specialized analytical methods for diagnosis. Establishing physiological ranges for oxidative stress biomarkers is crucial in ruminant medicine to identify a clear threshold for this condition (Celi, 2011).

Estimating the total antioxidant capacity might provide a solution to the challenge posed by the presence of numerous antioxidants, as it reflects the combined effect of all antioxidants present in animal (Abdel-Saeed & Salem, 2019). Furthermore, TAC offers valuable insights into the dynamic balance between pro-oxidants and antioxidants in the plasma compartment (Ghiselli *et al.,* 2000).

Nutritional deficiency or imbalance is observed in different cities in Egypt and worldwide. in a previous study in Menoufia Governorate, the authors reported significant reductions in serum zinc, copper, vitamin A, ALP , and SOD while significant increase in MDA in sheep suffered from skin diseases associated with nutritional deficiency signs including anorexia, emaciation, easily detached wool, alopecia, diarrhea, pasty feces, nervous signs, and parakeratosis (Abo Amer et al., 2020).

Moreover, (Ibrahim *et al.,* 2017) conducted a survey in Qena governorate involving 300 sheep of various ages and sexes. Diseased sheep showed signs such as of diarrhea, emaciation, loss of appetite, dehydration, alopecia, and anemia with paleness of visible mucous membrane, which were linked to deficiencies in serum minerals including Zn^{2+}, Cu^{2+}, Fe^{2+}, Ca^{2+}, P^{3+}, and magnesium, besides reduction in RBC count and Hb concentration, influenced by factors such as sex and age. Furthermore, Study of (Galbat *et al.,* 2021), conducted in different localities in New Valley governorate found, reduced levels of serum Zn^{2+}, Cu^{2+}, Fe^{2+}, Ca^{2+}, P^{3+}, magnesium, albumin, RBC, and Hb concentration in sheep suffered from easily detached wool, anorexia, and loss of skin rigidity and elasticity. Furthermore, Soil analysis showed decreased Zn^{2+} and Cu^{2+} while increased iron and P^{3+} concentrations in certain areas. Water sample analysis revealed low concentrations of Zn^{2+}, Cu^{2+} and manganese in all studied areas.

However, there is no recently published data about nutritional deficiency in sheep in El-Minia governorate. Our study was performed to investigate alterations in haemato-biochemical constituents, mineral status, vitamin A levels, and oxidative stress markers (TAC) in sheep suffering from nutritional deficiencies. In addition, identifying the associated risk factors such as sex, age, health status, and mineral content in soil and water.

MATERIALS AND METHODS

1. Ethical approval:

All study procedures during sample collection were conducted in accordance with El-Minia University Animal Ethics Committee guidelines with approval number IRB-FVM-MU-2024-108. This research was carried out under stringent protocols to protect and ensure the welfare of the animals, avoiding any form of suffering or stress.

2- Animals

The present study was conducted on 150 sheep of different ages and sexes (lambs under 1 year old and adults of both sexes (male and non-pregnant female) aged approximately 2 ± 0.5 years old in the period from August 2022 to September 2023. Animals were selected from Abo-korkas center in El-Minia governorate, Egypt.

All sheep were admitted to case history and clinical examination (vital parameters and physical examination). The data of each examined animal was documented in a separate case sheet. Sheep were divided into two groups: a diseased group of 90 sheep (30 males, 30 females, and 30 lambs) exhibiting signs such as anorexia, easily detached wool, nervous symptoms, diarrhea, emaciation, paleness of visible mucous membrane, pica and alopecia, and a control group of 60 healthy sheep (20 males, 20 females, and 20 lambs) in good body score, physically and clinically healthy.

3-Clinical examination of sheep

Every animal underwent a comprehensive physical assessment, starting with a distant observation and followed by a detailed examination as mentioned by (constable *et al.,* 2017).

4-Samples

Blood samples were obtained from the jugular veins of both diseased and control animals according to (Pugh, 2002). These samples were split into two parts: The first part (2 mL) of blood was collected in EDTA tubes for hematological analysis. The second part (8mL) was collected in plain vacutainer tubes which were kept at 4 °C overnight and then centrifuged at 1000 rpm for 10 minutes. The sera were removed from the tubes, placed into Eppendorf tubes, and stored at -20°C until used for biochemical analysis. The clear, non-hemolyzed serum samples were analyzed in three parts: the first part assessed levels of zinc, copper, iron, calcium, phosphorus, urea, creatinine, ALT, AST, albumin, and total protein; the second part evaluated vitamin A levels, and the third part measured total antioxidant capacity (TAC).

Soil samples were gathered to estimate the available concentrations of microelements including (Cu^{2+}, Zn^{2+}, and Fe^{2+}) and macro elements including (Ca^{2+} and P^{3+}). Samples were collected from a depth of 25-30 cm in each area where the animals were fed. Three soil samples were taken, sealed in double-thick plastic bags, and stored in a cool, dark environment until they were analyzed. Furthermore, three water samples were obtained from the primary water source**.** Sterile, clear one-liter bottles were utilized to collect the water samples, which were then sent to the laboratory for analysis of various minerals, including Zn^{2+}, Cu^{2+}, Fe^{2+}, and Ca^{2+}.

5-Hematological analysis:

Hematological parameters were analyzed by the CBC Analyzer (KT-6400, China). The analysis included measuring of Hb concentration, RBC count, WBC count, PLT count, HCT %, LYM%, NEU %, and MID%.

6-Biochemical analysis

Biochemical kits were obtained from Spectrum company, Germany and Bio-Diagnostic company, Egypt and analyzed by spectrophotometer. The concentration of Zn^{2+}, Cu^{2+}, Fe^{2+}, Ca^{2+}, P^{3+}, Creatinine, urea, albumin, and total protein were estimated by colorimetric method following the guidelines provided by the manufacturers of the kits. in addition, ALT and AST were also determined by kinetic commercial kits. vitamin A level was assessed through using a semi-micro method of Neeld & Pearson (Kumar *et al.,* 2021; Vasquez-Gomez *et al.,* 2024).

7-Soil analysis

Mineral concentration in soil was assessed following the guidelines of (Cottenie *et al.,* 1982). The results were compared with the reference values of (Donia & Ibrahim, 2013., Galbat *et al.,* 2021; McDowell., 2003; pueyo *et al.,* 2005). Soil analysis was done in the National Research Centre (NRC), Cairo Governorate.

8-Water analysis

Mineral concentration in water was assessed by using computerized atomic absorption (GBC-932-AA) in the National Research Centre (NRC), Cairo Governorate. The results were compared with the reference values of international standard for drinking water (WHO, 1971).

9-Statistical analysis:

The data was analyzed using ANOVA with the GLM procedure from SAS (SAS Institute, 2009). Significant treatment effects were further examined with Duncan's multiple-range test, considering differences significant at $P < 0.05$. Additionally, the Pearson correlation coefficient was calculated for certain parameters.

RESULTS

1-Clinical examination

The clinical examination of diseased sheep (n=90) in Abo-Korkas revealed that 10 cases (11.12%) showed anorexia, 25 cases (27.78%) showed easily detached wool, 6 cases showed nervous manifestations, and 5 cases showed pale mucous membranes (6.67% and 5.56%, respectively). Additionally, 21 cases (23.34%) of alopecia, 5 cases of diarrhea, and 15 cases of emaciation were reported (5.56% and 16.67%, respectively). Furthermore, 27 cases (30%) of pica were noted (Table 1 & Figure 1). Additionally, assessments of vital parameters and physical examinations, including measurements of body temperature, pulse rate, respiratory rate, and ruminal movement, indicated that all examined sheep fell within normal ranges as defined by reference values of (Constable *et al.,* 2017).

Table 1: Clinical findings in examined sheep.

Clinical signs of diseased sheep	Diseased sheep	
	Numbers	%
Anorexia	10	11.12
Easily detached wool	25	27.78
Nervous signs	6	6.67
Diarrhea	5	5.56
Emaciation	15	16.67
Paleness of visible mucous membrane	5	5.56
Pica	27	30
Alopecia	21	23.34

Alopecia and emaciation	Easy detached wool and history of lameness	Depigmentation of wool and history of pica

Figure 1: Clinical findings in sheep with nutritional deficiencies.

2-Hematological analysis

The results were classified based on animal age and sex to identify the impact of associated risk factors related to nutritional deficiency disease (Table 2).

1. Lambs

Hematological parameters showed no significant variations in diseased lambs, compared to the control group (P>0.05)

2. Adults

A. Males

In diseased males, there was a significant decrease in red blood cell (RBC) count and hemoglobin (Hb) concentration relative to the control group (*P < 0.05)*. However, no significant differences were observed in hematocrit (HCT %), white blood cell (WBC) count, lymphocyte percentage (LYM %), mid-sized cell percentage (MID %), neutrophil percentage (NEU %), or platelet (PLT) count when compared to the control group *(P > 0.05)*.

B. Non-pregnant Females

Non-significant changes were detected in any of the hematological parameters when comparing the diseased female group to the control group (P > 0.05).

Table 2: Hematological parameters values in control and diseased sheep.

Parameters	Group	Abo-korkas center		
		Male	Female	Lamb
RBC (10^6/ul)	Control	9.02±0.16	8.67±0.51	8.22±0.17
	Diseased	7.85±0.19*	8.96±0.26	7.94±0.21
Hb (g/dl)	Control	11.67±0.33	9.23±0.07	10.67±0.24
	Diseased	8.80±0.29*	9.50±0.51	10.03±0.15
HCT (%)	Control	25.53±1.64	23.42±1.42	26.60±0.87
	Diseased	23.67±0.33	23.63±1.05	23.77±0.96
WBC (10^3/ul)	Control	15.37±0.54	16.74±1.76	22.88±0.26
	Diseased	14.21±1.07	17.19±1.54	20.77±1.96
LYM %	Control	85.50±1.59	72.52±6.12	78.37±3.46
	Diseased	84.30±2.55	84.57±4.42	88.20±5.37
MID %	Control	8.73±2.12	14.18±2.01	15.77±4.80
	Diseased	11.00±0.32	10.12±3.10	7.37±2.37
NEU %	Control	7.57±3.24	5.30±4.80	5.87±1.47
	Diseased	4.70±2.31	5.32±1.88	4.20±3.15
PLT (k/ul)	Control	357.00±22.50	441.50±49.56	490.33±30.17
	Diseased	368.33±10.48	430.33±67.80	539.00±3.79

Values are presented as means ± SE. * Denotes significant changes from control when *$P < 0.05$.*

3-Minerals, Vitamin A, and TAC

The results were classified based on animal age and sex to identify the impact of associated risk factors related to nutritional deficiency disease (Table 3).

1. Lambs

There was a significant decrease in the levels of Zn^{2+}, Cu^{2+}, and total antioxidant capacity (TAC) in the diseased lambs compared to the control group ($P < 0.05$). In contrast, there were no significant changes observed in the levels of Fe^{2+}, Ca^{2+}, P^{3+}, and Vitamin A in the diseased lambs when compared to the control group ($P > 0.05$).

2. Adults

A. Males

Diseased males exhibited a significant decrease in Zn^{2+}, Cu^{2+}, Vitamin A, and TAC levels compared to healthy males ($P < 0.05$). On the other hand, there were no significant changes observed in Fe^{2+}, Ca^{2+}, and P^{3+} levels in diseased male when compared to the control group ($P > 0.05$).

B. Non-pregnant Females

Diseased non-pregnant females showed a significant decrease in Zn^{2+} and Ca^{2+} levels compared to the control group ($P < 0.05$). While there were no significant changes observed in Cu^{2+}, Fe^{2+}, P^{3+}, Vitamin A, and TAC levels in diseased female group when compared to the control group ($P > 0.05$)

Pearson's correlation coefficient indicated that Zn^{+2}, Cu^{+2}, and Vitamin A were positively correlated with TAC in all blood serum samples (Table 4).

Table 3: Concentrations of selected minerals, Vitamin A, and TAC in control and diseased sheep.

Parameters	Group	Abo-korkas center		
		Male	Female	Lamb
Zn^{2+} (µg/dl)	Control	88.67±2.03	94.17±4.53	100.33±2.91
	Diseased	73.00±3.21*	77.47±3.98*	70.67±3.48*
Cu^{2+} (µg/dl)	Control	99.67±2.03	96.17±3.64	100.00±2.89
	Diseased	80.33±0.88*	86.33±2.75	72.33±3.93*
Fe^{2+} (µg/dl)	Control	88.67±1.86	142.67±5.41	133.33±6.01
	Diseased	86.67±1.20	95.17±7.34	81.67±4.18
Ca^{2+} (mg/dl)	Control	8.00±0.21	8.55±0.15	8.07±0.38
	Diseased	7.70±0.26	7.17±0.28*	7.27±0.15
P^{3+} (mg/dl)	Control	2.97±0.79	3.68±0.21	3.90±0.40
	Diseased	3.53±.09	3.40±0.12	3.57±0.18
Vitamin A (IU/dl)	Control	69.67±1.45	65.08±0.99	67.00±1.15
	Diseased	63.67±0.88*	60.08±2.87	59.00±2.31
TAC(Mml/l)	Control	1.50±0.03	1.48±0.01	1.67±0.02
	Diseased	1.40±0.02*	1.47±0.04	1.39±0.02*

Values are presented as means ± SE.* Denotes significant changes from control when *P <0.05*.

Table 4: Correlation coefficient (r) among Zn^2, Cu^2, Vit A and TAC in serum samples of sheep.

			Zn^{2+}	Cu^{2+}	Vit. A	TAC
Male (n=50)	TAC	R	0.73^{s*}	0.69^{s*}	0.72^{s*}	1.000
Female(n=50)	TAC	R	0.38^{m*}	0.73^{s*}	0.42^{m*}	1.000
Lamb (n=50)	TAC	R	0.94^{s*}	0.95^{s*}	0.94^{s*}	1.000

Pearson correlation coefficient (*r*) Between 0 and 1 = Positive correlation. ^{S}= strong Strength (*r* > 0.5), ^{m} = moderate Strength (*r* Between .3 and .5). *= Significant *P <0.05*.

4-Biochemical parameters

The results were classified based on animal age and sex to identify the impact of associated risk factors related to nutritional deficiency disease **(Table 5).**

1-Lambs

Total protein levels were significantly lower in diseased lambs compared to the control group (*P < 0.05)*. In contrast, there were no significant changes in the levels of albumin, ALT, AST, urea and creatinine between the diseased and control groups (*P >0.05)*.

2-Adults

A. Males

Total protein levels were significantly reduced in diseased males in comparison to the control group (*P <0.05)*. In contrast, there were no significant changes in the levels of albumin, ALT, AST, urea and creatinine between the diseased and control groups (*P >0.05)*.

B. Non-pregnant Females

Total protein levels were significantly lower in diseased females when compared to the control group (*P < 0.05)*. In contrast, there were no significant changes in the levels of albumin, ALT, AST, urea and creatinine between the diseased and control group (*P >0.05)*.

Table 5: The values of serum biochemical parameters in control and diseased sheep.

Parameters	group	Abo-korkas center		
		Male	Female	Lamb
Albumin (g/dl)	Control	3.57±0.12	3.50±0.27	3.17±0.03
	Diseased	3.83±0.28	3.27±0.22	3.43±0.22
TP (g/dl)	Control	7.30±0.06	7.57±0.29	6.37±0.12
	Diseased	6.23±0.09*	6.42±0.06*	5.77±0.09*
ALT (U/L)	Control	20.55±1.02	21.86±1.17	23.43±2.47
	Diseased	18.90±0.46	21.45±1.56	20.87±1.48
AST (U/L)	Control	20.83±1.12	21.17±0.81	22.37±1.39
	Diseased	23.67±0.33	22.30±0.99	22.57±0.81
Urea (mg/dl)	Control	36.00±1.15	45.17±1.78	43.00±3.61
	Diseased	36.33±0.88	38.33±3.54	39.33±2.60
Creatinine (mg/dl)	Control	1.17±0.03	0.98±0.08	1.20±0.06
	Diseased	1.08±0.07	0.83±0.02	1.30±0.06

Values are presented as means ± SE.* Denotes significant changes from control when *$P < 0.05$.*

5-Soil analysis

Soil analysis results indicated low concentrations of Zn^{2+}, Cu^{2+}, and Ca^{2+}, while the concentration of Fe^{2+} was high. Finally, P^{3+} levels showed normal concentration compared to normal reference values (Table 6).

Table 6: Extractable mineral concentrations in soil at Abo-korkas center in El-Minia governorate, Egypt.

		Zn^{2+} (ppm)	Cu^{2+} (ppm)	Fe^{2+} (ppm)	Ca^{2+} (mEq/100g)	P^{3+} (ppm)
Abo-korkas (n= 3)		0.4^{L}	0.2^{L}	11.2^{H}	1.5^{L}	14.78^{N}
Reference values	L	< 1.0	< 0.5	< 4.0	< 2.4	< 10.0
	N	1.0-2.0	0.5-1.0	4.0-6.0	2.4-5	11-15
	H	> 2.0	> 1.0	> 6.0	> 5	> 15

Values denoted with superscripts are categorized as follows: (L) indicates values lower than the reference values, (N) represents values within the normal reference range, and (H) signifies values higher than the reference values. The results were compared with the reference values of (Donia & Ibrahim, 2013., Galbat *et al.,* 2021; McDowell., 2003; pueyo *et al.,* 2005).

6-Water analysis

Water analysis results indicated low concentrations of Zn^{2+}, Cu^{2+}, Fe^{2+}, and Ca^{2+} compared to normal reference values (Table 7).

Table 7: Mineral concentrations in water at Abo-korkas center in El-Minia governorate, Egypt.

	Zn^{2+} (mg/L)	Cu^{2+} (mg/L)	Fe^{2+} (mg/L)	Ca^{2+} carbonate (mg/L)
Abo-korkas (n= 3)	$<0.1^{L}$	$<0.1^{L}$	0.1^{L}	24^{L}
Critical levels	3.0	2.0	0.3	500

Values with superscripts (L) are lower than the critical levels of international standard of drinking water (WHO, 1971).

DISCUSSION

Minerals and vitamins are crucial micronutrients needed in small amounts to promote essential biological processes for cell metabolism in livestock. Sufficient intake of these micronutrients is vital for optimal wool, meat, and milk production in sheep (Lee *et al.,* 2002).

In our study, the clinical signs of affected sheep with nutritional deficiency were anorexia, easily detached wool, nervous symptoms, diarrhea, emaciation, and alopecia. Similar clinical signs were recorded by other literatures (Abo Amer *et al.,* 2020; Ibrahim *et al.,* 2017).

Moreover, other signs including pica and paleness of mucous membrane which similar to those recorded by (Ebrahim, 2015; Salem, 2017). Furthermore, our study identified nervous manifestations such as ataxia and lameness linked to disturbances in the musculoskeletal system. These symptoms align with findings by (Mobarak.,1998), who noted that deficiencies in manganese, zinc, and copper were associated with locomotor issues and lameness, accompanied by swelling in the hock and knee joints.

In a previous study (Ebrahim, 2015), the authors concluded that multiple trace elements deficiencies particularly Cu^{2+}, Fe^{2+}, and $Zn^{2+}$are the common cause of pica.
Reduced levels of cytochrome C and lysyl oxidase cause poor growth. Additionally, Cu-Zn dismutase, which is involved in the production of melanin, is the main cause of alterations in wool (Rucker, 2007)

Iron, zinc, and copper deficiencies may be the cause of ill-thriftness and anemia, which is indicated by paleness of visible mucous membranes (Radostits *et al.,* 2000).

Lower zinc levels are linked to decreased animal growth, dermatitis and impaired animal ability for utilizing of food (Harvey, 2008).

In our investigation, we identified that reduction in vitamin A was simultaneous with occurrences of alopecia and wool loss. Similar results were obtained by (Everts *et al.,* 2013).

Our study revealing variations in haemato-biochemical constituents results in sheep based on age (adults vs. young), sex and health status.

Research of (Ibrahim *et al.,* 2017) indicated that factors like sex, age, lactation, and pregnancy influence mineral deficiencies. Besides, (Dar *et al.,* 2014) noted that age, sex, production level, activity level, species, and genetics affect mineral needs and deficiencies. Notably, younger animals typically absorb minerals more efficiently than adults.
In this study there were argumental results related to hematological alteration in nutritionally deficient sheep, some results showed significant reduction, others recorded no changes.

In the current research, the RBC count and Hb concentration align closely with those reported by (Ibrahim *et al.,* 2017). They noted that in sheep with trace mineral deficiencies, there were no significant changes in RBC counts between healthy and diseased female sheep and lambs. However, significant decreases in RBC counts were observed in diseased male sheep and elder ewes compared to healthy counterparts. Others detected non-significant changes in Hb concentration and RBC counts (Nelson *et al.,* 1984). On contrast, significant reductions in RBC counts and hemoglobin were recorded in other reports (Abd El-Raof & Ghanem, 2006; Salem, 2017).

The reduction in erythrogram (RBCs count and Hb concentrations) may be the result of an interference with the normal metabolism of iron, as insufficient copper inhibits iron absorption, releases iron from body reserves and uses iron in hemoglobin formation

(Church and Pond, 1988). Also, Insufficient copper leads to a decrease in copper-containing cytochrome oxidase within mitochondria, which in turn slows down the reduction of iron and ultimately reduces the synthesis of "heme" molecules that use iron (Harvey, 2008).

The mean values of total WBCS count and differential leucocytic count showed non-significant changes between healthy and diseased sheep. These results coincided with those of (Abd El-Raof & Ghanem, 2006; Ebrahim, 2015; İçen *et al.,* 2008).

Biochemical findings of serum minerals of diseased sheep showed a significant reduction in Zn^{2+} and Cu^{2+}, while Fe^{2+} showed insignificant changes between diseased and control groups, Identical results recorded by (Ali, 2000; Ebrahim, 2015; Fahmy *et al.,* 1980) observed significant reduction in iron, zinc, and copper in sheep with alopecia and pica. Despite this (Akgül *et al.,* 2000) revealed that the iron levels of healthy and diseased sheep (showed signs of alopecia and pica) did not differ significantly.

Minerals, particularly copper and zinc, have an impact on wool. They can do this via interfering with the intake of food, rumen function, or by directly affecting the metabolism of sheep (Freer and Dove, 2002).

Calcium and inorganic phosphorus levels showed non-significant changes in lamb. And this result was nearly similar to those of (Ndlovu *et al.,* 2009), who found that in healthy control animals, the blood serum minerals magnesium, calcium, and phosphorus generally decreased with age beyond one year. A possible reason is that younger animals have a higher absorption rate of dietary elements (such as calcium, phosphorus, and magnesium) than older ones (Gurgoze & Icen, 2010).

In addition, serum inorganic phosphorus showed a non-significant change between control and diseases male and female group which agreed with the findings of (Ebrahim, 2015; İçen *et al.,* 2008).

On the other hand, our results also showed a significant change in calcium levels in the female group which agreed with (Venjakob *et al.,* 2017) revealed that the calcium level in their investigation was lower than that of the control group (the emaciated animals had about half of the control animals' levels).

Decreases in calcium and phosphorus can also harm the herd's general health and cause low activity. Acute recumbency, reduced mobility, diminished performance, and weight loss (Zhang *et al.,* 2016).

Furthermore, (Ibrahim *et al.,* 2017) mentioned that changes in the Ca: P ratio could prevent the pituitary gland from acting. As a result, the gastrointestinal tract's ability to absorb phosphorus, manganese, zinc, copper, and other elements is impaired.

In our study, vitamin A levels showed a significant reduction in diseased male sheep that exhibited signs such as alopecia, wool loss, and poor coat quality. These results are consistent with the findings of (Everts *et al.,* 2013; Hensel, 2010).

Vitamin A deficiency is linked to epithelial cell differentiation, metaplasia, xerophthalmia, anorexia, and decreased immunity. This is because vitamin A is necessary for normal differentiation of cells and development, which is vital for the transduction of light to the neural signals required for vision (Green *et al.,* 2012).

Reduction in serum level of TAC was recorded in our study. TAC measures the antioxidant capacity of all antioxidants in a biological sample (Kusano & Ferrari, 2008; Omur *et al.,* 2016). Assessing TAC at the herd level could provide a more precise and dependable measure of animal welfare. Furthermore, TAC can be used to evaluate animals' nutritional status year-round or to compare the nutritional effects of various diets on animals (Celi, 2011). Moreover, TAC, and total oxidant status (TOS) are widely used markers in the determination of oxidative stress in ruminants (Celi, 2010).

Oxidative stress exhibits a disruption in the equilibrium between the antioxidant system and free radicals (Yang & Li, 2015., Yoshika & Naito, 2002) and contributes to the damage of various macromolecules, such as DNA, RNA, cholesterol, lipids, carbohydrates, and proteins. The oxidation of these macromolecules produces measurable end-products, which help in assessing oxidative stress levels (Celi and Gabai, 2015).

The present study showed that Zn^{+2}, Cu^{+2}, and Vitamin A were positively correlated with TAC. This is due to the role of zinc and copper in the antioxidant enzymatic mechanism, specifically in the activity of copper-zinc superoxide dismutase (Abo Amer *et al.,* 2020). Furthermore, Vitamin A plays a significant role in enhancing immunological response, reducing oxidative stress (Jin *et al.,* 2014), and strengthening antioxidant defense mechanisms to prevent oxidative damage (Kleczkowski *et al.,* 2004) Moreover, (Beattie & Kwun, 2004) reported that zinc deficiency developed endothelial cells susceptibility to oxidant stress. In addition (Lai *et al.*, 1994) recorded that Oxidative stress is expected in copper-deficient sheep because there was a significant reduction in serum Cu-Zn SOD associated with a substantial reduction in copper levels thus, in sheep deficient in copper, oxidative stress is predicted. This is consistent with the findings of (AL-Rikabi & Jawad, 2013), which indicate that copper, an antioxidant, plays a key role in reducing and avoiding oxidative stress.

Supplementing dairy cows with a high dosage of Vitamin A significantly reduces serum malondialdehyde (MDA) levels and enhances the activities of antioxidant enzymes such as GPx, SOD, CAT, and total antioxidant capacity (Jin *et al.,* 2014). Besides, (Elshahawy & Aly, 2016) reported that serum MDA level showed significant increase while TAC, zinc, copper and iron levels showed significant decrease in cattle affected by Pica.

Regarding to protein profile, serum total protein was significantly reduced in diseased groups of males, females and lambs without significant changes in albumin level. Those results agreed with (Ebrahim, 2015; Nelson *et al.,* 1984). The cause of total protein reduction was attributed to copper deficiency which causes inappetence (Salem, 2017).

The results of the current research recorded non-significant changes in ALT, AST, BUN and creatinine, similar to previous studies (Ebrahim, 2015; İçen *et al.;* 2008)

One of the goals of this research is to establish the link between blood parameter alterations (hematology, biochemistry, and TAC) in sheep and its relation to the surrounding environment as soil and water or related to other causes

Based on soil and water analysis results (Table 6&7), we anticipate that the deficiency of minerals in the soil and water has a direct effect on serum minerals deficiency in sheep. (Zeleke, 2021) recorded that imbalances of minerals (deficiencies or excesses) in soils and forages are frequently attributed to low production and reproductive problems among grazing ruminants. Poor body conditions, slow body weight gain, low fertility, and high mortality are normally observed in mineral-deficient animals. A strategic mineral survey, including soil, water, grass, and animal tissue analysis, is recommended to identify mineral imbalances and proper supplement.

The present results are nearly similar to (Shen & song, 2021) who concluded that Cu deficiency in sheep is primarily caused by inadequate Cu concentrations in the soil and forage. Cu deprivation in forage disrupted blood parameters such as physiology, biochemistry, immunity, and antioxidant levels. Furthermore, (Song & Shen, 2020) recorded that Zinc deficiency in soil and feed is believed to cause sheep diseases and negatively impacts the antioxidant system, growth and development, and can lead to many animal diseases.

CONCLUSIONS

Our findings confirmed a significant relationship between nutritional deficiencies in sheep and alterations in haemato-biochemical constituents, mineral status, vitamin A levels, and oxidative stress markers (TAC), influenced by factors such as sex, age, health status, and mineral content in soil and water. Therefore; it is advisable to include both macro and microelement supplements in the diet to guard against any suspected deficiencies in the diet, soil, and water.

CONFLICT OF INTERESTS

The authors declared that no conflict of interest exists.

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الدراسات الإكلينيكية والمعملية عن نقص التغذية في الأغنام في محافظة المنيا، مصر

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هدفت هذه الدراسة إلى الكشف عن التغيرات في المكونات الهيماتو- كيميائية، والمعادن المختارة ،وفيتامين أ،والسعة الكلية لمضادات الأكسدة في الأغنام التي أظهرت علامات نقص غذائى،وتحديد عوامل الخطرالمرتبطة به. شملت هذه الدراسة ١٥٠ من الغنم من أعمار وأجناس مختلفة (حملان تحت سن السنة الواحدة وأغنام بالغين من كلا الجنسين الذكر والأنثى تتراوح أعمارهم بين ٢ ± ٠,٥ سنة) في مركز أبو قرقاص في محافظة المنيا، مصر. خضعت جميع الأغنام لفحوصات سريرية ومخبرية. تم تقسيم الأغنام إلى مجموعتين: مجموعة مرضية تضم ٩٠ من الأغنام تظهر بها علامات مثل تساقط الشعر، وسهولة انتزاع الصوف، وإنحراف بالشهية، ومجموعة ضابطة تضم ٦٠ من الأغنام السليمة. تم جمع عينات من الدم والماء والتربة من أغسطس ٢٠٢٢ إلى سبتمبر ٢٠٢٣. أشارت النتائج المتعلقة بالمجموعات المختلفة من حيث العمر والجنس إلى انخفاضات كبيرة في مستويات الهيموجلوبين، وعدد كريات الدم الحمراء، والزنك ، والنحاس، والكالسيوم، وفيتامين أ، والسعة الكلية لمضادات الأكسدة، والبروتين الكلى فى المجموعة المريضة مقارنة بالمجموعة الضابطة.أظهرت تحليلات التربة إنخفاض تركيزات الزنك ، والنحاس ، والكالسيوم ، بالإضافة إلى ارتفاع في تركيز الحديد، بينما كشفت عينات المياه عن مستويات منخفضة من الزنك ،والنحاس ،والحديد، والكالسيوم. تشير النتائج إلى وجود علاقة كبيرة بين نقص التغذية في الأغنام والتغيرات في المكونات الهيماتو- كيميائية، والمعادن المختارة،وفيتامين أ، والسعة الكلية لمضادات الأكسدة، والتي تؤثر عليها عوامل مثل الجنس ، والعمر ، والحالة الصحية ، ومحتوى المعادن في الماء والتربة.