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Field deficiency of macro and microelements is associated with alterations in hematology, hepatic and kidney functions and electrocardiography in sheep

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ABSTRACT

The objective of this work was to study the impact of deficiency of certain macro and microelements on clinical, hematobiochemical and electrocardiographic changes in sheep. The survey study was carried out on a total of 300 sheep, of which 250 suffered from mineral deficiency and 50 apparent healthy were used as control. Clinical investigations of diseased sheep revealed change wool color, easily detached wool, alopecia, pale mucous membrane, and diarrhea. Clinical examination showed significant elevation ($p< 0.05$) of respiratory rate and heart rate. Hematological examination revealed significant ($P<0.05$) decrease in Hb, RBCs, WBCs and HCT, with increase in MCV, MCH. Biochemically, there was a statistically significant ($P<0.05$) decline in Ca, Mg, P, Cu, Zn, Se, Fe, TP and albumin, whereas ALT, ALP, LDH, cTnI, and creatinine levels increased significantly ($P<0.05$). Electrocardiographic examination showed different changes, including abnormal T-waves (wide and enlarged), premature ventricular depolarization and cardiac arrhythmia. It was concluded that macro- micro element deficiency is associated with clinical, biochemical, hematological and ECG alterations. In addition, they have been related to dysfunctions of internal organs including liver, heart and kidney. Therefore, it is recommended to prescribe a proper feeding management program containing the necessary macro- and micro elements for sheep flocks and do not rely only on the grazing as a main source of feed to maintain the health and production of sheep.

Introduction

Small ruminants are widely used due to their various advantages, including little financial outlay, having quick fattening and breeding cycles. Compared to cattle, his offers a quicker return on investment, helping with short-term financial flows (Jemberu *et al*., 2022). The total number of small ruminants in Egypt is 30,784,085 according to latest figures available from the FAOSTAT (2020) (Benfield *et al*., 2023). The most important factor to give good performance, production, reproduction and prevent wasting in sheep herds is nutritional management which offers sufficient intake of all important minerals, vitamins, and micronutrients (Asín *et al*., 2021).

Small ruminant depended mainly on grazing fodder throughout the year. Thus, animal nutrient intake is tightly tied to the kinds and quantities of nutrients delivered by grassland. Grass is incapable of providing all the nutrients required for proper animal growth and metabolic processes, particularly minerals (Moyano Tapia *et al*., 2020).

Minerals are required for healthy nutrition, where the proper concentration of mineral elements allows for the satisfactory growth because of their physiological, catalytic, functions and regulatory function (Suttle, 2010). Minerals are divided into macro-minerals and micro-minerals (Radwinska and Zarczynska, 2014).

Calcium (Ca), phosphorus (P), potassium (K), sodium (Na), chloride (Cl) and magnesium (Mg) are examples of macrominerals. While Micro-minerals or trace elements include iron (Fe), zinc (Zn), copper (Cu), and selenium (Se) (Bednarek and Bik, 1994). The proper proportions of these important mineral elements are critical in the maintenance of numerous biochemical processes in animals (Larrán *et al*., 2021).

Mineral deficiency can occur in animals as result of poor feed quality, reduced mineral absorption or assimilation in the body or if adequate availability of vital mineral nutrients is not supplied (Radwinska and Zarczynska, 2014; Jin *et al*., 2023)

Mineral deficiency could be primary where the minerals are deficient in the diet or secondary where the minerals could not be absorbed or metabolized inside the body (Baugreet *et al*., 2017).

Anemia, loss of appetite, emaciation, diarrhea, dehydration, and pale mucous membranes are most common in sheep which exhibited a decrease in serum level of Ca, Mg, Fe, Cu, Zn, and P (Ibrahim *et al*., 2017). A drop in serum Zn content caused physiological problems as well as a variety of illnesses such as anorexia, weight loss, delay development, skin lesions, eyelid swelling, loss of wool, and dermatitis (Sloup *et al*., 2017), on other hand Joint stiffness and a decrease in hematological markers (Song and Shen, 2020). Reduction of serum levels of Cu resulted sheep suffered from anemia, poor development, wool keratinization, and bone diseases (Hefnawy and El-Khaiat 2015; Mandour *et al*., 2021). While reduced Ca levels are commonly associated with retarded growth and milk production, swollen joints, tremors, and general lethargy (Masters and White, 1996).

Troponins are proteins have regulatory and structural functions in both cardiac and skeletal muscles. The cardiac isoform of TnI (cTnI) varies from the two other TnI isoforms present in skeletal muscle by 32 amino acids at its N-terminus. This property of cTnI makes it the gold standard in determining cardiac injury (Karapinar *et al*., 2010; Fartashvand *et al*., 2013). Nutritional restriction associated with increased level of cTnI as mentioned by Tümer *et al*. (2021).

Na, K, and Ca concentrations are crucial for appropriate depolarization and repolarization of heart muscle. Change in the concentration of these electrolytes may lead to abnormal ECG pattern. For example, dis-

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turbance in K and Mg levels are associated with abnormalities of T wave morphology. Ca concentration can affect the conduction system marked by prolonged periods in ECG traces while Mg deficiency could affect the atrial and ventricular contractility and fibrillations (Johri *et al*., 2009; Yousif, 2015; Varshney, 2020).

Therefore, the present survey study was conducted to investigate the alterations in hematology, hepatic and kidney function, and ECG in sheep affected with macro- and micro-deficiency under natural conditions.

Materials and methods

Animals

A total of 300 Balady sheep from both sex (200 female and 100 male), different ages (250 ≥ 2 years and 50 ≤ 2 year) and different physiological condition (150 non pregnant and 50 pregnant) from different private flocks of sheep at Qalioubia Governorate, Egypt, were included in the current survey study. The source of feed for sheep included in this study was grazing only without any proper feeding management system. Sheep were divided into two groups. The first group (control group) included 50 apparently healthy sheep based on physical examination (normal body temperature, pulse, respiration rates, shiny eyes without discharges, no abnormal lung sounds on auscultation, raised head, normal posture and appetite, no diarrhea or lameness). The second group (diseased group) included 250 sheep suffered from wool loss, change in color of wool, decreased body weight, and pale mucous membrane and other clinical signs. The epidemiological data of sheep under study are shown in Table 1.

Ethical approval

All studies were carried out after approval from the ethical and animal care and use committees in Faculty of Veterinary Medicine at Benha University with the ethical number BUFVM 17-02-23.

Clinical examination of the animals

The clinical examination was conducted and recorded for the detection of clinical signs that related to mineral deficiency (Radostits *et al*., 2007).

Samples

A jugular venipuncture was used to obtain ten milliliters of blood from each animal. Blood sample was divided into two parts; the first half without anticoagulant was used to separate serum while the other half was added to EDTA to collect whole blood. All samples were quickly chilled on crushed ice and sent to the laboratory for further processing. The EDTA blood was used for CBC; while those in plain tubes were stored overnight at room temperature until they were centrifuged at 3000 r/min for 15 minutes. Only clear sera were collected, aliquoted, and stored at -20°C for further biochemical analysis.

Hematological analysis

A hematological analyzer (Mindray BC_3000 plus Edan H30) was used to assess total erythrocytic count (RBCs), hemoglobin (Hb), packed cell volume (PCV), total leukocytic count (WBCs), and differential leukocytic counts, as well as platelets.

Biochemical analysis

To quantify, the following commercial kits were utilized in accordance with the providers' normal methodology: Cu (SIGMA-ALDRICH Co, USA), Zn (Abnova Co, Taiwan) and Fe (Abcam Co, UK), Se (Abbexa, UK), total protein, albumin, urea and creatinine (Gamma Trade Company, Egypt). Calcium (BioMed, Egypt, REF: CAL103100), phosphorus and magnesium (Bio-Diagnostic, Giza, Egypt). Sodium, potassium, chloride, ALT (alanine aminotransferase), ALP (alkaline phosphatase) and LDH (lactate dehydrogenase) levels (Spectrum Company, Egypt) on a selective chemistry analyzer (Apple 302, USA) and cardiac troponin I (MyBioSource, USA).

Electrocardiographic examinations

The ECG was recorded with the base apex lead system II and limb lead (Ghanem, 1997; Koether *et al*., 2016). ECGs waves were recorded using a single channel electrocardiographic equipment (BTL-08 SD ECG, IndustriesLtd.161 Cleveland Way, Stevenage, SG1 6BU UK) Using a paper speed of 25mm/s and calibration of 10 mm equal to 1 mV. All waveform amplitudes and durations were estimated by hand, with each little square representing 0.02 ms on the vertical plane and 0.1mv on the horizontal plane.

Statistical analysis

The present data was statistically analyzed using SPSS program version 23 by comparing between the two studied groups means by independent T test). A difference between control group and diseased group was considered significant at $P < 0.05$.

Results

Clinical findings

Clinical findings of examined animals are divided into; clinical signs related to wool which included change of color (83.33%), steely wool (83.3%) easily detached wool (20%) and alopecia (15%). General condition and GIT disturbance which included diarrhea 10.66%, pale mucous membrane, dullness and inapptance (30%), as showed in Table 2 and Fig. 1. The body temperature showed non-significant changes between diseased and control group while there is significant ($P<0.05$) increase in pulse rate and respiratory rate between diseased and control sheep as showed in Table 3.

Fig. 1. Emaciated ram with knuckling on fetlock joint.

Table 1. Animal epidemiological date.

	Age		Sex		Pregnancy	
	≥ 2years	≤ years	Females	Males	Pregnant	Not pregnant
Total number	250	50	200	100	50	150
Percentage	83.30%	16.67%	66.66%	33.33%	25%	75%

Table 2. Frequency of clinical findings in examined sheep.

Clinical sign	Number	%
Change in wool color	250	83.33%
Easily detached wool	60	20%
Alopecia (partial loss of wool)	30	15%
Steely wool (matted together)	250	83.30%
Diarrhea	32	10.66%
In appetence (partial loss of appetite)	90	30%
Dullness	90	30%
Emaciation	80	26.67%
Unable to stand on hind quarter	7	2.33%
Knuckling	5	1.60%
Wobbling of hind quarter	35	11.66%
Pale mucous membrane	90	30%

Hematological changes

The current data showed significant decreases (P<0.05) in Hb, total RBCs, HCT and WBCS in diseased sheep compared to control one with significant (P<0.05) increases in MCV, MCH, MCHC and platelet count in diseased than control sheep as shown in Table 3.

Table 3. Temperature, respiratory, heart rate and hematological parameters (mean±SE) of control sheep and deficient sheep.

Parameters	Control sheep (n=50)	Deficient sheep (n=250)	p-value
Temperature (°C)	39.1±0.03	39.2±0.07	0.68
Pulse rate (Beats/min)	83.2±2.2	141.7±6.5*	0.00
Respiratory rate (Breaths/min)	19.3±1	27.2±1.5*	0.00
RBCs (x10^6/ul)	7.3±0.1	5.3±0.3*	0.00
Hb (g/dl)	11.8±0.5	8.6±0.6*	0.00
HCT (%)	36.0±1.6	23.7±0.6 *	0.00
MCV (fl)	34.7±0.08	51.4±9.4	0.1
MCH (pg)	9.0±0.4	19.1±1*	0. 001
RDW-CV (%)	11.9±0.1	15.6±0.7*	0.00
MCHC (g/dl)	33.1±0.2	54.1±3*	0.00
Platelet (x10^3/ul)	157.8±29.7	434.3±44.3*	0.00
WBCs (x10^3/ul)	11.9±0.7	6±0.5*	0.00
Segmented (%)	29.8±1.4	43.6±1.8 *	0. 001
Lymphocyte (%)	58.2±2	46.3±2.5*	0.00
Monocyte (%)	1.7±0.1	4.5±0.5*	0.00
Eosinophil (%)	2.0±0.01	4.0±0.2*	0.00

*Values with an asterisk within the same raw are statistically significant (P<0.05). WBCs (white blood cells), Hb (hemoglobin), RBCs (red blood cells), HCT (hematocrit), MCV (mean corpuscular volume), MCHC (Mean corpuscular hemoglobin concentration).

Changes in Macro-, micro-minerals and electrolytes

Biochemical analysis of serum mineral and electrolyte revealed significant (P<0.05) reduction of serum Ca, P, Mg, Cu, Fe, Zn, Se, Na and Cl in diseased sheep in comparing to control one. On the hand, K showed a significant (P<0.05) increase in diseased group compared to control group as shown in Table 4.

Changes in serum liver, heart and kidney functions

When compared to the control group, the blood liver enzymes and kidney function of deficient sheep exhibited a significant (P<0.05) increase in ALT, ALP, LDH, cTnI, and creatinine. While urea showed non-significant change compared to control sheep (Table 4). The total protein and albumin were significantly (P<0.05) decreased in affected sheep group compared to control group (Table 4).

Table 4. Mineral and biochemical profiles (mean±SE) of control sheep and deficient sheep.

Parameters	Control sheep (n=10)	Deficient sheep (n=50)	p-value
Ca (mg/dl)	8.6±0.1	6.4±0. 1*	0.00
P (mg/dl)	5.6±0.3	3.4±0.1*	0.00
Mg (mg/dl)	3.0±0.1	2.0±0.03*	0.00
Na (mEqu/l)	172.7±2.2	141.7±4.2*	0.00
K (mEqu/l)	3.6±0.2	5±0.1*	0.00
Cl (mEqu/l)	104.2±2.2	77.8±3.2*	0. 001
Cu (ug/dl)	121.8±3.4	93.1±1.4*	0.00
Zn (ug/dl)	109.3±9	77.4±4*	0.00
Fe (ug/dl)	119.1±3.9	74.8±4*	0.00
Se (ug/dl)	106.1±5.9	62.3±3.1*	0.00
Total protein (g/dl)	7.6±0.4	5.4±0.4*	0.02
Albumin (g/dl)	3.7±0.3	2.3±0.1*	0.01
ALT (U/l)	35.5±3.4	57±4.6*	0.02
ALP (U/l)	71.3±14.4	130.3±11.6*	0.03
LDH (U/l)	77.3±6	169±24.3*	0.02
Urea (mg/dl)	27.3±4.9	38±5.5	0.22
Creatinine (mg/dl)	0.4±0.07	0.9±0.1*	0.02
cTnI (ng/ml)	0.1±0.02	0.3±0.06*	0.02

*Values with an asterisk within the same raw are statistically significant (P<0.05). Ca (calcium), P (phosphorus), Mg (magnesium), Na (sodium), K (potassium), Cl (chloride), CU(copper), Zn(zinc), Fe(iron), Se(selenium), ALT (alanine aminotransferase), ALP (alkaline phosphatase) and LDH (lactate dehydrogenase), cTnI(cardiac troponin)

The results of electrocardiographic (ECG) examination

The ECG trace of control sheep showed normal heart rhythm as recorded in all cases Figure 2. On the other hand, the diseased sheep showed some numerous arrhythmias such as sinus tachycardia, sinus arrhythmia, atrial fibrillation, ventricular premature contraction, myocardial weakness, and bradycardia Figures 3, 4.

Analysis of the ECG results for diseased sheep showed significant (P<0.05) increases of QRS amplitude and T-wave duration and amplitude and showed a significant (P<0.05) decrease of P wave duration and amplitude, QRS duration and Qt interval compared to control group while P-R interval show decrease but non-significant as shown in Table 5.

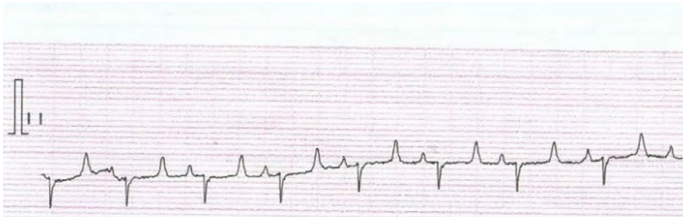

Fig. 2. ECG trace of healthy sheep of control group using base-apex lead system showed normal ECG trace. The heart rate of diseased sheep is 89 beat /min. Trace is recorded at a paper speed of 25mm/sec and calibration of 10mm/mV.

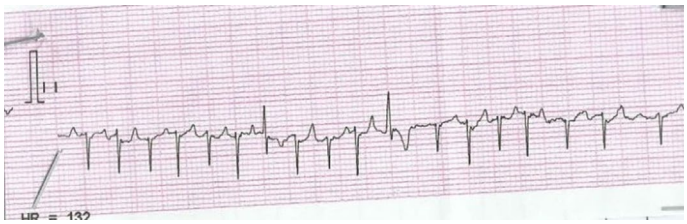

Fig. 3. ECG trace of diseased sheep using base apex lead system showing premature ventricular depolarization in two waves, tachycardia and increase QRS amplitude and duration is decreased. The heart rate is 132 beat/min. recorded at a paper speed of 25mm/sec and calibration of 10mm/mV.

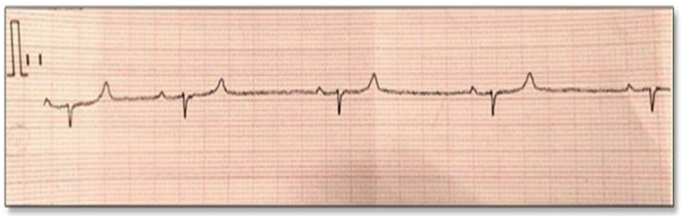

Fig. 4. ECG traces sheep using base-apex lead system showing bradycardia (decrease of QRS amplitude and duration & increase RR interval). The heart rate is 55 beat /min. Trace is recorded at a paper speed of 25mm/sec and calibration of 10mm/mV (1 cm= 1 mV).

Table 5. ECG changes (mean±SE) of control sheep and deficient sheep.

Parameters	Control sheep(n=10)	Deficient sheep (n=100)	p-value
T-wave amplitude (mV)	0.3±0.01	0.5±0.01*	0.00
T-wave duration (s)	0.02±0.001	0.04±0.004*	0.00
P-wave amplitude (mV)	0.12±0.006	0.1±0.005*	0.00
P-wave duration (s)	0.02±0.001	0.01±0.009*	0.00
QRS- amplitude(mV)	0.6±0.02	0.8±0.05*	0.00
QRS- duration(s)	0.02±0.001	0.01±0.001*	0. 001
PR interval (s)	0.07±0.003	0.08±0.04	0.69
QT interval (s)	0.1±0.001	0.1±0.002*	0.00

*Values with an asterisk within the same raw are statistically significant (P<0.05).

Discussion

Nutritional problems are regarded as one of the most important problems affecting animal output. The most important factor in preventing wasting in sheep herds is nutritional management. Feed must be supplied in sufficient amount and of high-quality in order to provide appropriate consumption of all required minerals, vitamins, and micronutrients (Asín *et al*., 2021).

Minerals are essential in biological and physiological processes (Nwosu, 2019). Mineral deficit may result from reduced mineral feed concentrations, absorption, and bioavailability (Arshad *et al*., 2021).

The clinical findings in examined animals were change in wool color, steely wool, alopecia in some area in body and easily detached wool. These findings are similar to clinical findings observed by other authors (Ebrahim 2015; El-khaiat *et al*., 2012; Constable *et al*., 2016; Saleh, 2019). Generally, rough hair coat condition and/or wool abnormalities were associated with copper, zinc, and selenium deficiency. Cu deficiency leads to low tyrosinase activity (Hefnawy and El-Khaiat, 2015) that resulted incomplete sulfhydryl group oxidation in prekeratin and diminished melanogenesis that clinically appear on animal as poor wool quality and hypopigmentation thus explained by Mauldin and Peters-Kennedy (2016). Zn deficiency leads to Wool fibers loses their crimp, becoming thin and loose, and the entire fleece may shed as reported by Song and Shen (2020) and explained by Ogawa *et al*. (2016) as Zinc promotes cellular integrity, epidermal cell development, keratin synthesis, wound healing. Besides, help in development and differentiation of the epidermal keratinocyte.

In the present study, diseased sheep showed inappetence, emaciation and diarrhea this result agreed with (Abo Amer *et al*. 2020; Wu *et al*., 2020). Diarrhea is a common clinical symptom in secondary copper insufficiency linked with molybdenosis and previous studies explained that diarrhea in sheep occur when level of Cu, Zn and cobalt reduced to (0.13 ppm, 0.77 ppm, 0.39 ppm) respectively (Kaneko *et al*., 2008).

Physical examination of sheep revealed that no significant change in body temperature when compared affected with control one this result is the same as that founded by Saleh (2019) as mineral deficiency did not cause elevation of body temperature due to absence of inflammatory condition.

The increased respiratory and heart rate in the present study coincided with those reported by previous investigators (Abd El-Raof and Ghanem, 2006; Naji, 2017; Saleh, 2019). Previous studies (Kusiluka and Kambarage, 1996) explained that copper or zinc deficiency cause hypoxia and anemic conditions thus lead to increase respiratory and pulse rates as a compensatory mechanism.

Diseased sheep in this study had decreases in Hb, total RBCs, HCT and WBCS than control animals, a result which is similar to El-khaiat *et al*. (2012); Mohammed *et al*. (2013); Ebrahim (2015) and Ibrahim *et al*. (2016). The decrease hematology parameters could be related to a disruption in iron metabolism, as copper shortage reduces iron absorption, iron release from body storage, and iron utilization in hemoglobin formation (Abd El-Raof and Ghanem, 2006; Ibrahim *et al*., 2017). On the other side, this reduction might be due to a decrease in the ceruloplasmin enzyme in serum (Hefnawy and El-Khaiat, 2015), which responsible for transfers iron from storage cells in the colon and liver to transferase in plasma. Transferase is an enzyme that delivers iron to bone marrow for Hb synthesis. Moreover, reducing iron liberation from normally damaged erythrocytes (Kaneko *et al*., 2008; Ebrahim, 2015). Or may attributed to Zn deficiency as explained by Ibrahim *et al*. (2016) who reported that low RBCs, HB, and WBCs with induced Zn deficiency may return to impaired cell replication and protein synthesis, and consequently the formation of blood cells. (Huo *et al*., 2020) reported that anemia in sheep induced by selenium shortage is caused by an excessive breakdown of circulating erythrocytes as well as a lack of adequate formation of mature erythroid cells.

Current data showed a significant reduction of serum Ca, Fe, P, Mg, Cu, Zn and Se. Those results were similar to those recorded by Saleh (2019) who found significant decrease in Ca, P, Mg, Cu and Zn level in affected sheep. In other study, (Ebrahim, 2015) found a significant decrease in Cu, Zn and Fe in sheep suffer from alopecia but non-significant changes in Ca and p levels in diseased and normal sheep.

Low serum level of examined mineral may attributed to decrease feed intake, or feeding on inferior quality feed as most of the survey was conducted on sheep flock graze randomly without proper feeding management system. On the other side significant decrease of Cl serum level explained by increase reabsorption of bicarbonate and increase excretion of Cl in renal cell (Kumar *et al*., 2018). Ca and P are essential minerals in the ruminant body. Reduction in feed intake and hypoalbuminemia could be the cause of low level of Ca in the present study. About 40-45% of excreted calcium is bounded with albumin mainly (Mansour, 2006; Faez *et al*., 2013). The considerable drop in serum phosphorus concentrations appeared to be due to decreased phosphorus absorption from the intestine and tissue phosphorus resorption (Saleh and Allam, 2014).

Low level of P is associated with decrease of feed intake, bone softness, lameness and acid base imbalance regarded as an essential element in the cellular energy, bone structure within the animal body and a vital factor of sustaining a proper balance of acid and base. Reduced bone density, increased fracture risk and osteoporosis risks accompany marked with low Ca level (Masters and White, 1996; Theobald, 2005).

Development of low level of Mg could be attributed to hyperkalemia. Several studies showed that sheep feed ration high in K suffered from hypomagnesaemia (Castillo *et al*., 2016).

Cu insufficiency is more prevalent in ruminants. Cu shortage in sheep can be primary, resulting from lower intake, or secondary, resulting from altered absorption, decreased tissue availability, or increased excretion by interaction with other with dietary molybdenum (Mo) and Sulphur in ruminants, resulting in the creation of molybdates and thiomolybdates, which bind Cu and reduce its uptake and utilization ((El-khaiat *et al*., 2012; Asín *et al*., 2021).

Zn, being one of the necessary mineral elements, is crucial in a variety of living activities. Zn is necessary for the production of metallothionein and gene regulatory proteins (Jin *et al*., 2023). Zn deficiency may be primary owing to decreased ration levels or secondary due to the presence of a chemical interfering with its absorption or metabolism, despite normal food concentrations (Ibrahim *et al*. 2017).

Present study showed reduction in iron level which resulted most of hematological parameters have decreased. This is consistent with Yatoo *et al*. (2013) who indicated that Fe deficit affected on sheep performance and productivity. low level of iron in this study could related to cu deficiency that regulate absorption and utilization of iron from storage cell and usage in hemoglobin synthesis by action of ceruloplasmin this explained by Sharma *et al*. (2005) and Kaneko *et al*. (2008)

Serum Se deficient was discovered among all important mineral elements that examined. Se required for effective antioxidant defense, thyroid function, reproduction, immunity, and health in ruminant (Jin *et al*., 2023).

Serum biochemical characteristics are linked to a variety of physiological activities, including the performance of animals and metabolism of lipids. These metrics can be used to assess animals' nutritional condition, tissue damage, and organ malfunction (Liang and Yan, 2020). Liver enzymes and renal function of mineral deficient animal showed increased ALT, ALP, LDH and creatinine in comparison to control group. While urea showed non-significant change compared to control group agreed with result obtained by Abo Amer *et al*. (2020) and Huo *et al*. (2020). In addition, Jin *et al*. (2023) mentioned that ALT, AST, ALP, albumin, and TP can be used to assess animals' nutritional condition, tissue damage, and organ malfunction. ALT accumulates mostly in the cytoplasm of hepatocytes, where its activity is roughly 3,000 times that of serum. As a result, when the liver is injured, the damaged hepatocytes produce ALT, dramatically increasing serum ALT activity (Liu *et al*., 2014).

Nutritional deficiency associated with decrease antioxidant capacity and increase oxidative stress thus led to damage of internal organs and tissue this explained by Naji (2017). As increased free radical lead to damage hepatocytes this significantly enhances the serum ALT activity.

Reduction level of Ca, Zn Cu and Se in sheep associated elevation level of ALP as observed by Jin *et al.* (2023). ALP is useful bone mineralization indicator (Brichacek and Brown (2019). LDH extracellular appearance is employed to identify cell damage or death. In cardiac, hepatic, skeletal muscular and renal disorders, levels are elevated (Klein *et al.*, 2020). A lack of dietary necessary mineral compounds may adversely affect the sheep's muscle mass or myocardial (Jin *et al.*, 2023). This may attribute to increase oxidative stress and destruction of internal organs in case of nutritional deficiency Naji (2017).

Regarding to Total protein and albumin significant ($P<0.05$) decrease in affected mineral deficient group than control group this result agrees with Abd El-Raof and Ghanem (2006) and Huo *et al.* (2020) who reported that decrease level of albumin, it signals protein insufficiency in animals, but it might also be due to a reduction in albumin production induced by liver illness. While disagree with Jin *et al.* (2023) who reported that mineral deficiency associated with increase in total protein and albumin and attributed that damage of hepatocyte in case nutritional deficiency.

Result of Cardiac marker showed significant increase in cardiac troponin I compared to control as showed in Table 4. This result agrees with Tümer *et al.* (2021) which reported that hypocalcaemia in ewe cause myocarditis which associated with significant increase in all cardiac marker include cTnI, LDH and CK and explained this increase due to increased permeability of the cell membrane of cardiac myocytes. Additionally, Cu deficiency in goat cause abnormal increase in cTnI and LDH. This mentioned by Mandour *et al.* (2021) who reported that cu deficiency in goat cause myocardial damage and increase level of cTnI and returned this damage due to decrease antioxidant capacity and increase oxidative damage of myocardium due to lower activity of SOD and ceruloplasmin as cu play major in their structure. Moreover, Low level of selenium in sheep and goat kids lead to increase level of in cTnI as resulted by Ataollahi *et al.* (2013) and Fakour *et al.* (2017).

Based on the findings of Başbuğan *et al.* (2010), cTnI concentration in ruminants as a reference value, the mean value of cTnI in sheep was 0.15 g/L, with a range of 0.0-0.21 g/L. not affected by sex or age this agree with our result.

Non-invasive auxiliary technologies like electrocardiogram (ECG) have become repeatable and practical for identifying cardiovascular issues in farm animals in the field. The ECG is a critical diagnostic tool for assessing cardiac conductivity efficiency. As a result, wave time, voltage, and arrhythmias may be more properly comprehended (Fakour *et al.*, 2017; Kocatürk *et al.*, 2019).

ECG in ruminants is largely used to diagnose heart arrhythmia, conduction abnormalities, and electrolyte imbalances. This will aid in determining the prognosis of heart illness and therapy options (Devadevi *et al.*, 2022).

The result of ECG of mineral deficient animal showed a significant ($P<0.05$) increase of T wave duration and amplitude and QRS amplitude while showed a significant ($P<0.05$) decrease of P wave duration and amplitude, QRS duration and Qt interval in comparison to control group while PR interval show decrease but non-significant. This result agreed with Fakour *et al.* (2017) who resulted that Serum cTnI concentration measurements can aid in the diagnosis of kids with Se deficiency. ECG changes in Se deficient animal include one or more type or arrhythmia in young lambs this indicated that ECG can be useful in aid diagnosis of selenium deficiency.

Also agreed with Mandour *et al.* (2021) who reported that deficiency of Cu in goat produce significant reduction in T wave and P wave time while cause increase QRS time but not agree in decrease amplitude of T wave and non-significant increase in QRS amplitude.

For normal cardiac depolarization and repolarization, the concentrations of Na, K, and Ca are critical. Any variation in the concentration of these electrolytes might result in an abnormal ECG pattern as hyperkalemia and hypomagnesaemia associated with to large spiked "T" wave, Flat or absence of "P" wave and Sino ventricular rhythm while Hypocalcaemia lead to Prolonged Q-T interval as it correlated with Ca concentration. Hypomagnesaemia increased excitability of Purkinje fibers & associated with Ventricular tachycardia, Supraventricular tachycardia, atrial fibrillation &Ventricular fibrillation (Johri *et al.*, 2009; Yousif, 2015; Varshney, 2020).

Conclusion

Field conditions are usually associated with multiple mineral deficiencies in sheep. The deficiency of macro and micro-elements is related to ovine liver, heart and kidney dysfunctions and ECG changes. However, we could not determine the detrimental effect of each deficiency alone because mixed deficiencies commonly occur under field condition. Therefore, it is recommended to prescribe a proper feeding management program containing the necessary macro- and micro elements for sheep flocks and do not rely only on grazing as a main source of feed to maintain the health and production of sheep.

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Conflict of interest

The authors declare that they have no conflict of interest.

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