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Gene Expression of Oxidative/Antioxidative Markers, VDR, CAMK4 and Ceruloplasmin in Baladi Sheep a with Minerals Deficiency

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Abstract

THE OBJECTIVE of this work was to elaborate gene expression and serum profile of oxidative/antioxidant and bone markers associated with minerals deficiency and their correlation with mineral deficiency in sheep. The study was carried out on 26 selected diseases cases out of survey on 250 sheep suffered from mineral deficiency compared to 10 apparent healthy control sheep based on clinical examination. Biochemically, there was a significant ($P<0.05$) decline in Ca, P, Mg, Cu, Zn, Se, Fe, TAC, GPx, SOD, CAT, OC and CP, whereas MDA level was significantly increased ($P<0.05$). Gene expression of SOD, CAT, GPX1, VDR, CAMK4 and ceruloplasmin genes were significantly down-regulated ($P<0.05$) in diseased sheep. A contrary tendency was evoked by the gene OXSR1.The variability in studied genes alongside alterations in the serum profile of investigated markers could be a reference guide for limiting the mineral deficiency in sheep through prescribing an efficient nutritional management strategy for sheep flocks that includes the essential macro- and micro aspects, and not depend just on grazing as a primary source of feed to sustain sheep health and output.

Keywords: Gene expression, micro, macro-element, oxidative/antioxidant markers, bone marker, sheep.

Introduction

Sheep are regarded as vital agricultural animals since they are necessary for human populations to survive[1]**.** This need can be successfully met by improving these animals' capacity for reproduction and output [2]. Trace mineral deficiencies could have an impact on small ruminant performance; productivity and involved in several biological functions, such as energy production, collagen formation, cell metabolism maintenance, oxygen transportation, hormone production, enzyme activity and vitamin synthesis especially in substandard pastures or traditional methods [3]**.** Reduced levels of trace elements are typically linked to a number of negative outcomes, such as irregularities in immune, metabolic, hormonal, and reproductive processes [4]**.** It could have happened as a result of a poor diet, poor absorption, or a metabolic process issue [5]. Previous studies showed that calcium (Ca), zinc (Zn), copper (Cu), iron (Fe) and selenium (Se) frequently lack in grazing sheep which raised on grassland [6]**.** Ruminants require mineral elements for nutrition and proper concentration ensures their growth, physiological and regulatory functions [7]**.** Ataxia, pale mucous membranes, diarrhea, loss of wool and color changes in the wool are clinical indicators of a mineral deficit in sheep [8].

Minerals and other micronutrients are crucial parts of the antioxidant defense system that protects tissues from damage caused by free radicals in order to maintain health [9]**.** The presence of mineral elements in blood significantly impacts the activity of antioxidant enzymes, thereby deficiency of mineral elements reducing their antioxidant effectiveness [10]**.**

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Consequently, it lead to the breakdown of the dynamic balance between antioxidant compounds and oxygen-free radicals [11].

The component of the antioxidant system, which protects an organism from free radicals, consists of both enzymatic and non-enzymatic mechanisms[12];[13] . The antioxidants enzymes that comprise the enzymatic system are mostly glutathione peroxidase GSH-Px, superoxide dismutase (SOD) and catalase (CAT). Additionally, the primary components of the non-enzymatic system are vitamin, cysteine, glutathione, and mineral elements [13]. Zinc, copper and selenium elements are used in the creation of antioxidant enzymes [9]. According to [10] zinc is a key component of the body's antioxidant system, which helps in prevention of cell membrane oxidation and reduce super anions and cations production. Furthermore, Cu is an important component of numerous enzymes, including superoxide dismutase (SOD) and ceruloplasmin; hence a lack of Cu impairs the operation of these enzymes [14]. Also Se in sheep is required for adequate antioxidant defense, thyroid function, reproduction, immunity and health so deficiency of se has been associated with oxidative stress, myodegeneration, hepatic degeneration and immunosuppression [15]. Deficiency in Cu and Zn is associated associated with increased Malondhyde dehydrogenase (MDA) level that leads to increase free radical which plays a role in damage of different internal organs[16,17,18].

Abd Ellah Argues that a deficiency in one antioxidant does not necessarily mean that all antioxidant defense systems are diminished; this is because of antioxidants work synergistically to neutralize oxidative stress[19]. As a result, researchers have developed various techniques for estimating total antioxidant capacity, with recent studies focusing on gene expression of antioxidant markers [20].

Serum Osteocalcin (OC) is one of bone turn over marker in sheep giving an indication about osteoclast cell activity during bone formation [21]. The bone markers osteocalcin and alkaline phosphatase were useful for identifying changes in bone length caused by variations in feed restriction levels in lamb [22]. Vitamin D regulates the homeostasis of calcium. Since the vitamin D receptor (VDR) mediates the benefits of vitamin D, down regulating VDR results in calcium shortage [23];[24][25]. Calmodulin is crucial for the Ca signaling system, controlling gene expression, metabolism, and facilitating calcium absorption through calcium-triggered Calcium/ calmodulin dependent protein kinase (CaMKK), mediated by CAMK1 and CAMK4 [26].

Given the importance of nutrigenomics, the influence of essential macro and micronutrients on animal genomes and subsequently their health is receiving remarkable attention [27]. Considering variations in biochemical indicators are dependent on specific gene transcripts, studies of gene expression evaluation in response to dietary regimens are conducted [28]. So far, there is limited information about the oxidative/antioxidant, bone marker alterations and gene expression associated with sheep mineral deficiency. Therefore, the aim of the present study was to evaluate the oxidative/antioxidant, bone marker alterations associated with sheep mineral deficiency by exploring gene expression and serum profile of investigated markers in Baladi sheep.

Material and Methods

Animals and study design

A total of 26 Baladi sheep from both sexes with range of age 6 months - 2 years and a range of body weight 30-40 kg, were selected from a total 250 examined sheep included for the current study based on clinical examination. The experiment was carried out in 2022-2023, in private flocks of Baladi sheep at Qaluobia Governorate. The sheep were divided into two groups as following: Control group (CG): 10 healthy sheep and mineral deficient group (DG): 26 sheep suffering from macro and micro mineral deficiency. The investigated sheep were subjected to clinical examination including recording of temperature, pulse, respiratory rates and mucous membranes [29]. All procedures were performed in accordance with the guidelines of Faculty of Veterinary Medicine (Banha University) (Approval No BUFVM 17-02-23), and approved by their Ethical Committees.

Blood sampling

Ten milliliters of blood was collected from each animal via jugular venipuncture. The collected blood was divided into two halves, the first half to collect serum without anticoagulant while the other half added to EDTA to collect whole blood. All samples were cooled on crushed ice and were transported immediately to the laboratory for further processing. The EDTA blood was used for real time PCR, while those in plain tubes were kept overnight at room temperature and centrifuged at 3000 r/min for 15 min. Only clear sera were collected then aliquoted and kept frozen at -20 C for subsequent biochemical analyses.

Biochemical analysis

The following commercial kits were used according to the standard protocol of the suppliers to quantify: GPx, CAT, SOD, TAC and MDA (Biodiagnostic, Egypt), the osteocalcin (OC), ceruloplasmin (Cp) levels were measured by Arbor Assays DetectX ® (USA) ® kits. Cu (SIGMA-ALDRICH Co, USA), Zn (Abnova Co, Taiwan), Fe (Abcam Co, UK), Se (Abbexa, UK), Ca (BioMed, Egypt, REF: CAL103100), P and Mg (Bio-Diagnostic, Giza, Egypt).

RNA extraction and reverse-transcriptase PCR

Whole blood samples from animals were subjected to total RNA extraction using Trizol™ reagent (Invitrogen, UK), in accordance with the manufacturer's instructions (Direct-zolTM RNA MiniPrep, catalog No. R2050). The amount of RNA extracted quantified and qualified using a NanoDrop® (ND-5000 spectrophotometer) and its integrity was evaluated by agarose gel electrophoresis. An equivalent to 1 mg of RNA was transferred to cDNA with high capacity (SensiFastTM cDNA synthesis kit, Bioline, catalog No. Bio- 65053). PCR amplifications were performed in a final volume of 20 µl containing total RNA template up to 1 µg, 4 µl 5× Trans Amp buffer, 1 µl reverse transcriptase and DNase free-water up to 20 µl. Reverse-transcription was done through placing the final reaction volume in a thermal cycler with the following cycling program; at 25°C for 10 min for primer annealing, followed by reverse transcription at 42°C for 15 min, then inactivation at 85°C for 5 min. The samples were held at 4°C [30].

Quantitative Real Time PCR

Relative quantification of mRNA level of SOD, CAT, GPX1, OXSR1, ceruloplasmin, VDR and CAMK4 markers was performed of each animal by real-time PCR using SYBR Green PCR Master Mix (2x SensiFastTM SYBR, Bioline, catlog No. Bio-98002). The primer sequence was designed according to the pubMed published sequence of *Ovis aries*. Primer sequences, annealing temperature and the size of each amplified PCR product are shown in **Table 1.** The house keeping gene *GAPDH* was used as an internal control. The reaction mixture was carried out in a total volume 20 µL consisted of 10 µL 2x SensiFast SYBR, 3 µL cDNA, 5.4 µl H2O (d.d water), 0.8 µl of each primer. The PCR cycling conditions were as follows: denaturation program 94°C for two minutes; amplification and quantification program repeated 40 cycles of denaturation temperature 94°C for 10 seconds, annealing temperature as displayed in Table 1 for 30 seconds, and extension temperature 72°C for 20 seconds. At the end of the amplification phase, a melting curve analysis was performed to confirm the specificity of the PCR product. The relative expression of the gene in each sample versus a control in comparison to *GAPDH* gene and calculated according to the $2^{-\Delta\Delta Ct}$ method [31].

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. Pearson's simple correlation method was used for determination of correlations between the molecular markers and biochemical parameters. A difference between control group and diseased group was considered significant at $P< 0.05$.

Results

Clinical examination

Clinically, normal body temperature, pulse, respiration rates, shiny eyes (no discharges), normal wet muzzle and muffle, no abnormal lung sounds on auscultation, raised head, normal posture and appetite, no diarrhea, alopecia and lameness were displayed by clinically healthy sheep. In contrast, deficient group displayed symptoms such as weakness, emaciation, depression, retardation of growth, change wool color, easily detached wool, partial alopecia and pale mucous membrane (Figs.1, 2, 3).

Biochemical profile of Macro-, micro-minerals, osteocalcin, Cp and oxidative/ anti-oxidative biomarkers

Biochemically, there was a significant ($P<0.05$) reduction of serum Ca, P, Mg, Cu, Fe, Zn, Se, Cp and OC in deficient sheep in comparing to control one (Table 2). Concerning the oxidative and anti-oxidative biomarkers, there was a significant ($P < 0.05$) reduction in the serum concentration of TAC, GSH-Px, CAT and SOD with a significant ($P < 0.05$) elevation in the serum concentration of MDA in deficient sheep compared to control group (Table 2).

Gene expression pattern of oxidative/antioxidant markers, VDR, CAMK4 and ceruloplasmin

The evaluated indicators' gene expression profiles are shown in Figs. 4 & 5. SOD, CAT, GPX1, VDR, CAMK4 and ceruloplasmin genes were significantly down-regulated ($P<0.05$) in diseased sheep than in the control ones. However, the expression level of OXSR1 was considerably greater in the diseased sheep. For every gene analyzed in the diseased sheep, OXSR1 exhibited the greatest level of mRNA (1.52 ± 0.15), while GPX had the lowest level (0.46 ± 0.06). The SOD exhibited the highest possible quantity of mRNA amongst all the genes inspected in the healthy sheep (1.54 ± 0.17), whereas OXSR1 had the lowest (0.56 ± 0.06).

Correlation between mineral serum level and biochemical parameters

Serum level of Ca, Mg, Cu, Zn and Fe had significant ($P<0.05$) positive correlation with serum value of Osteocalcin, Cp, GPx, SOD, CAT and TAC while show specific negative correlated with serum level of MDA. Serum P concentration had significant ($P<0.05$) inversed correlation with MDA, but had significant ($P<0.05$) positive correlation with Osteocalcin, Cp, GPx, CAT & TAC (Table 3).

Correlation between serum minerals level and gene expression

Table (4) showed correlation between serum mineral levels and gene expression pattern. The mRNA expression of SOD, GPX, Cp and CAMK4

gene had significant (P<0.05) positive correlation with serum level of Ca, Mg, Zn and Fe. The mRNA expression of CAT gene had significant (P<0.05) positive correlation with serum level of Ca, Cu, Zn and Fe. A significant (P<0.05) negative correlation was observed between mRNA level of OXSR1and serum level of Ca, P, Mg, Cu, Zn and Fe. The mRNA expression of VDR gene had significant (P<0.05) positive correlation with P, Cu and Se.

Correlation between Serum antioxidant and gene expression

Table (5) elucidated the correlation between biochemical parameters and their respective genes. The mRNA expression of SOD, CAT, GPX and Cp gene was significant (P<0.05) positively correlated with serum level of SOD, CAT, GPX and Cp, respectively. The mRNA expression of VDR gene had significant (P<0.05) positively correlated with serum level of Osteocalcin.

Discussion

One popular method for assessing the health of farm animals is the measurement of biochemical parameters [32]**.** Numerous physiological and pathological factors including stress, pregnancy, management, illnesses, diet, and environmental influences, may have an impact on these parameters' levels [33]**.** The mineral elements' has critical function include physiological, catalytic, and regulatory roles and sustenance of livestock's numerous metabolic activities[7] [34]**.** Sheep will suffer from mineral shortage if enough supply of vital mineral nutrients is not guaranteed [6]**.** Small ruminants depended on the conventional method or grazing on low quality pastures are more likely to suffer from mineral deficits [3]**.** The objectives of the study were to validate the use of gene expression and serum profile of major and trace elements, Cp, Oc and antioxidant markers alterations as diagnostic criteria for mineral deficiency in Baladi sheep.

Clinically the mineral deficient group had weakness, emaciation, retardation of growth, change wool color, easily detached wool, partial alopecia and pale mucous membrane, according to a clinical examination. The aforementioned clinical indicators were additionally detailed by [8,35]**.**

Two of the main minerals that make up a ruminant's body are Ca and P. In addition to being essential for preserving acid-base balance and being a major component of the energy skeleton in the body of an animal, they also play a significant role in neurotransmission and other metabolic processes [36]**.** Additionally, it's critical for protein synthesis and ruminal microbial fermentation [37]**.** In ruminants, hypophosphatemia and hypocalcemia are quite prevalent [38]**.** Since diet affects magnesium content and the primary mechanisms controlling its equilibrium are renal excretion and intestinal absorption, the study's found low serum concentration of magnesium may have resulted from feeding on low-quality feed [3]**.** The significant decrease of Ca, P and Mg in the present study was similar to previous studies [8]

The serum level of Zn, Cu, Fe and Se showed significant decrease in mineral deficient group when compared with healthy ones. These findings were in agreement with those given by[39];[8] who revealed that, in comparison to healthy sheep, the blood levels of copper, zinc, cobalt, magnesium, and calcium in the affected sheep were noticeably lower. Additionally, similar to [18] who found that, sheep which has alopecia, loss of wool around eye, pale mucous membrane had low value of zinc and copper than apparently health sheep. In a different investigation, the affected animals' serum concentrations of Zn, Fe and Se were shown to be lower than those of the healthy ones [4]**.** Moreover, [40] noticed that, sheep suffered from different degree of alopecia and wool eating showed significant decrease in serum level of Cu, Zn and Fe, while showed non-significant change in serum level of Ca and P.

Low blood levels of the studied minerals may be associated to reduced feed intake or dining on lower quality feed, since the majority of the survey was conducted on sheep flocks that were permitted to graze at random without an effective feeding management system [41]**.** However, the serum selenium concentration in the deficient sheep was below normal, and there were no signs of myodestructive illness. This is consistent with the findings of [3]**,** who reported that not all animals deficient in selenium develop the white muscle illness and that many of them never show clinical symptoms.

Iron is necessary for the synthesis of many other enzymes, including cytochrome enzymes of the electron transport chain, and is also necessary for the oxygen transfer to tissues, maintenance of the oxidative enzyme system, and the synthesis of ferritin, hemoglobin, and myoglobin [42]**.** This study's notable low iron levels could be the result of a copper deficiency and drop in blood levels of ceruloplasmin which is an enzyme that moves iron from the gut's and liver's storage cells to the transferase in plasma [8]**.** According to [43]**,** copper (Cu) increases the transfer of iron from tissues to plasma, iron absorption from the gastrointestinal tract (GIT) and iron incorporation into hemoglobin. The positive correlation in our study between iron, copper and ceruloplasmin has been proven by previous studies [44]**.**

SOD is a crucial part of the antioxidant system because it scavenges free radicals in animals, which allows it to oppose and prevent the harm that free radicals do to cells while also repairing the damage

over time [45]. Since Cu/Zn-SOD is the most prevalent of the four types of SOD, improving Cu/Zn-SOD production and synthesis can be achieved by raising the level of Cu *in vivo*[17][13]. The reaction product of SOD is the substrate of GSH-Px and CAT [17]. Furthermore, Glutathione functions as a protective substance by preventing the production of free radicals in cells, especially the intermediate components of reactive oxygen species. Thus, excessive levels of free radicals can lead to glutathione consumption [10]. Catalase is a crucial enzyme involved in antioxidant defense. Almost all aerobic organisms include it. Two molecules of hydrogen peroxide are broken down by catalase into one oxygen molecule [46]. The GSH-Px active site contains selenium, which is essential for catalyzing the reduction of hydrogen peroxide and other organic peroxides GSH-Px activity [35]. Zinc activates glutathione peroxidase, converting glutathione into oxidized form. The body produced more lipid peroxide and used less glutathione peroxidase when zinc was lacking, which led to a drop in the amount of active glutathione peroxidase [10].

Monitoring the oxidant/antioxidant status in the diseased animal revealed a significant increase in serum concentration of MDA with significant decrease in serum enzymatic activities of TAC, GSH-Px, CAT, SOD and Cp. These results were in line with those of [13], who found that a lower intake of Cu in the diet was linked to lower levels of GPx, SOD, and CAT and higher levels of MDA, as well as a decrease in the body's ability to create antioxidants. Conversely, increasing the amount of copper in the diet boosts the body's antioxidant capability. These results consistent with positive correlation between Cu serum level and serum level of GPx, SOD, CAT and Cp.

Furthermore, Naji found that deficiencies in zinc and copper are typically linked to elevated MDA levels and decreased levels of CAT and GPx, which raise free radical levels and contribute to damage to many internal organs[18]. Another study [10] revealed that sheep with zinc deficiency had significantly lower serum levels of SOD, CAT, GPx, and TAC, while their MDA levels were much higher. Zn is favorably linked with the levels of GPx, SOD, CAT, and TAC, which is consistent with our findings. Yet, there was a negative correlation with MDA. Selenium deficiency resulted in increasing increase the serum level of MDA, decrease level of GPx and CAT [35].

Tissue-specific copper-binding protein, or Cp, is expressed differently in various tissues. The protein Cp has a variety of uses. During development, Cp expression increases rapidly in the liver and lungs, and it eventually overrides the copper-binding protein in plasma [47]. Furthermore, oxidative tissue damage caused by inflammation is prevented by ceruloplasmin and SOD, two copper-dependent enzymes with anti-inflammatory qualities [43; 16]. Since almost 90% of Cu is present in ceruloplasmin [43; 48], the decreased level of Cp in this study is consistent with the results obtained by [44; 12; 13] These results support our own, which showed a favorable association between Cu and Cp as well as with Zn, Se, and Fe, all of which are thought to be among the body's most significant antioxidants. As a result, serum Cp, SOD, GSH-Px, CAT, and MDA are employed as useful markers for sheep mineral deficiencies.

Osteoblast activity in bone creation is indicated by biochemical markers such as osteocalcin and ALP, which are used to monitor bone cell growth and turnover [21] The diseased group's serum osteocalcin levels significantly ($P<0.05$) decreased compared to those in the control one. This conclusion is consistent with the findings of [22], who found that feed limitation was linked to low levels of P and osteocalcin in lambs, but not with his assertion that there was no discernible change in the Ca level.

Nutrients can have a significant impact on the degree of DNA methylation, particularly genome-wide methylation. The investigation of these mechanisms is a new area of study known as nutritional genomics. Zinc (in rat liver) and selenium (in rat liver and colon) deficiency may decrease methyl group utilization [49]. This, in turn, may result in histone hypomethylation and genomic DNA hypomethylation [27]. According to[50]zinc also affects transcription levels and gene expression. A decrease in Cu or Zn levels causes lipid peroxidation, which produces a lot of MDA, consumes a lot of SOD and GPx, and leads to a significant decrease in the content of SOD and GPx as well as the capacity to scavenge free radicals [51, 52].

There is low information about the role of mineral deficiency was responsible for gene expression of antioxidant enzymes. In the present study, the deficient sheep exhibited a significant down-regulation of SOD, CAT, GPX1, VDR, CAMK4, and ceruloplasmin genes compared to healthy ones. Meanwhile, the OXSR1 gene displayed an opposite pattern. To our knowledge the expression profile of SOD, CAT, GPX1, OXSR1, VDR, CAMK4 and ceruloplasmin genes and its association with mineral deficiency is scarcely reported in livestock. [44] Investigated the relationship between the expression of genes encoding cardiac copper chaperone and metrics of cardiac function in goats. The findings showed that the Cu deficient group had considerably lower relative mRNA expression of the genes for ATP7A, CTr1, LOX, and COX17 as well as ceruloplasmin (CP), troponin I3 (TNNI3), glutathione peroxidase (GPX1), and matrix metalloprotease inhibitor (MMPI1). Moreover, [26] elucidated the decreased expression of CAMK4 gene in blood of cows with hypocalcaemia. [53] Reported that the vitamin D

receptor (VDR) gene was significantly down-regulated in cows with both subclinical and symptomatic hypocalcaemia.

Oxidative stress-responsive kinase 1 (OXSR1) gene encodes OSR1, which controls downstream kinases in response to environmental stressors [54]**.** OXSR1 gene was up-regulated in endometritis Holstein cattle [55] and mastitic dromedary camels [56]**.** The observed alterations in the expression pattern of antioxidant and OXSR1 expression may be explained by a rise in oxidative damage resulting from a decrease in the levels of Ca and Cu, which in turn causes a decrease in SOD, CAT, and GPX [57]**;** [58]**.** Copper chaperone proteins maintain proper intracellular distribution and absorption of copper by mediating copper homeostasis inside the cell [59]. One of these proteins is ceruloplasmin (CP) [60],[43]. Cu deficiency in goat associated with low level of Cp mRNA gene expression [61] and this explained by [62] who reported that The liver's pre ceruloplasmin lacks the copper necessary to develop into ceruloplasmin. After being introduced into the bloodstream, pre ceruloplasmin quickly broke down.

The active form of vitamin D, 1,25-dihydroxyvitamin D3 (1,25(OH)2 D3), combined itself to its receptor protein, VDR gene and together they create a molecular complex that may either up- or down-regulate a number of genes, so impeding the maintenance of calcium homeostasis [23][25]**.** Our findings showed that the VDR gene is significantly down-regulated in deficient sheep, which may be a cause of the deficiency rather than a symptom.

Calmodulin performs a crucial part in the Ca signaling system, which controls gene expression and metabolism, promotes healthy cell growth and development, and transports calcium[26]. As such, one important function related to calcium is that it can enhances absorption via controlling transcription activators involved in inflammation and the immunological response through calcium/calmodulin-dependent protein kinase 4 (CAMK4) [63]**.** Dependence on Ca2+ leads to decreased expression of activated small calcium binding protein genes, affecting sarcoplasmic reticulum Ca2+ cycling, mitochondrial function, and reduced ROS production, potentially causing immune dysfunction.[64] This study revealed that deficiency of some micro and macro element has adverse effect on antioxidant capacity of sheep through decrease mRNA expression of (SOD, CAT, OXRS1 and GPx). In addition to, it has effect on bone as showed by decrease level of Osteocalcin, mRNA expression of VDR and CAMK4.

Conclusion

The results here in confirm that there were profound biochemical, antioxidant/ oxidant and bone markers alterations associated with ovine mineral deficiency particularly blood Ca, P, Mg, Cu, Zn, Fe, Se, Oc, Cp, SOD, CAT, GPx, TAC and MDA. Our findings highlight the significance of gene expression in investigated antioxidant/ oxidant and bone markers related genes as genetic markers for mineral deficiency in Baladi sheep. These findings suggest that variability in these genes could be used as proxy biomarkers for such disorder in Baladi sheep. The variable expression pattern of biochemical, antioxidant/oxiditve and bone markers related genes in resistant and non-resistant sheep to mineral deficiency could be a reference guide and a biomarker that can be used to follow up health status of sheep.

Statements & Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by RRE, MMG, YMR, HMK, AE and MAYH. The first draft of the manuscript was written by RRE and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request**.**

Consent to participate

Not applicable

Consent to publish

Not applicable.

TABLE 1. oligonucleotide primers sequence, annealing temperature and PCR product size of the studied genes.

Gene	Oligonucleotide sequence	Accession number	Annealing temperature (C^0)	Size (bp)
SOD	F5-TGATCATGGGTTCCACGTCC-3 R5-CACATTGCCCAGGTCTCCAA-3	NM_001145185.2	60	139
CAT	F5′-CAGTAGGAGACAAACTCAATG-3′ R5′- ACGACTCTCTCAGGAATTCTC - 3′	GQ204786.1	58	121
GPX1	F5′-GAGGAGATCCTGAATTGCCTGA-3′ R5′- ACCTCGCACTTTTCGAAGAGC- 3′	JF728302.1	60	95
OXSR1	F5- TGATGGTTGGAAGCCTTGCT-3 R5- TGTTCCCCCTTTGCCACAAT-3	XM_060402087.1	60	136
VDR	F5- TGTCCCCCTGCTCCTACAG-3 R5- CCGCTTGAGGATCATCTCCC-3	XM_042246802.2	60	203
CAMK4	F5′-CTTCTTCGCCTCTCACATCCA-3′ R5′- AGGTAAGCAACTGCCTCCAA-3′	XM_027970560.3	58	188
Ceruloplasmin	F5- TCACGATGCATGTGGGCAAT-3 R5-CATCCAGACTTGATCTCTTCGTTTG-3	AF134814.1	60	250
GAPDH	f5`- TGACCCCTTCATTGACCTTC-3` r5`- GATCTCGCTCCTGGAAGAG-3`	NM-001034034	60	143

SOD: super oxide dismutase, CAT: catalase, GPX1: glutathione peroxidase, OXSR1 oxidative stress-responsive kinase 1.VDR: Vitamin D receptor, CAMK4: Calcium/calmodulin dependent protein kinase 4.

TABLE 2. Biochemical profile (mean ±SE) of control sheep and deficient sheep.

Parameters	Control sheep (n=10)	Deficient sheep (n= 26)	p-value
Ca(mg/dl)	9.2±0.2	6.1 ± 0. 1*	0.001
P(mg/dl)	6.2 ± 0.3	3.06± 0.09*	0.001
Mg (mg/dl)	3.21 ±0.1	1.98 ± 0.03*	0.001
Cu (µ/dl)	126.54 ± 4.2	91 ± 1.08*	0.001
Zn(µ/dl)	121.4± 11.3	64.50 ± 3.12*	0.001
Fe(µ/dl)	118 ± 3.8	62.8 ±1.15*	0.001
Se(µ/dl)	111 ± 5.4	53.8 ± 1.9*	0.001
OC (ng/mL)	20 ± 0.9	9.3 ± 1.3*	0.003
Cp(µ/ml)	57.6 ± 3.1	30± 1.5*	0.02
GPx(µ/ml)	53.6 ± 2.4	22 ± 3.7*	0.002
SOD(µ/ml)	67.6 ± 4.6	27.3 ± 4.9*	0.004
CAT(µ/ml)	42.3 ± 2	15.3 ± 2.9*	0.002
TAC(µ/ml)	54 ± 3.2	28.3 ± 4*	0.008
MDA(nmol/L)	8.5 ± 0.9	25.9 ± 3.6*	0.01

*Values with an asterisk within the same raw are statistically significant (P<0.05).
Ca (calcium), P (phosphorus), Mg(magnesium), Cu(copper), Zn(zinc), Fe(iron), Se(selenium),
OC (osteocalcin), Cp (ceruloplasmin), GPx (glutathione peroxidase), SOD (superoxide dismutase), CAT (catalase), TAC (total antioxidant capacity) and MDA (Malondhyde dehydrogenase).

TABLE 3. Correlation between serum mineral level and serum antioxidant level.

Parameter		Ca	P	Mg	Cu	Zn	Fe	Se
Cp	Pearson Correlation	.921**	.958**	.815*	.841*	.961**	.973**	.919**
	Sig. (2-tailed)	.009	.003	.048	.036	.002	.001	.010
Osteocalcin	Pearson Correlation	.920**	.867*	.839*	.845*	.945**	.917*	.836*
	Sig. (2-tailed)	.009	.025	.037	.034	.004	.010	.038
GPx	Pearson Correlation	.956**	.879*	.930**	.927**	.908*	.930**	.877*
	Sig. (2-tailed)	.003	.021	.007	.008	.012	.007	.022
SOD	Pearson Correlation	.905*	.961**	.893*	.915*	.879*	.981**	.944**
	Sig. (2-tailed)	.013	.002	.017	.010	.021	.001	.005
CAT	Pearson Correlation	.981**	.897*	.948**	.944**	.907*	.936**	.910*
	Sig. (2-tailed)	.001	.015	.004	.005	.013	.006	.012
TAC	Pearson Correlation	.935**	.859*	.769	.781	.959**	.862*	.839*
	Sig. (2-tailed)	.006	.028	.074	.067	.002	.027	.037
MDA	Pearson Correlation	-.948**	-.913*	-.858*	-.879*	-.898*	-.877*	-.916*
	Sig. (2-tailed)	.004	.011	.029	.021	.015	.022	.010

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).
+ve mean positive correlation.-ve mean negative correlation

TABLE 4. correlation between serum mineral level and gene expression of oxidative/antioxidant markers, VDR, CAMK4 and oxidative/antioxidant markers, VDR, CAMK4.

		Ca	P	Mg	Cu	Zn	Fe	Se
SODgene	Pearson Correlation	.890*	.953**	.874*	.891*	.832*	.980**	.957**
	Sig. (2-tailed)	.018	.003	.023	.017	.040	.001	.003
CATgene	Pearson Correlation	.925**	.828*	.958**	.945**	.819*	.888*	.851*
	Sig. (2-tailed)	.008	.042	.003	.005	.046	.018	.032
GPXgene	Pearson Correlation	.792	.928**	.692	.732	.848*	.930**	.885*
	Sig. (2-tailed)	.060	.008	.127	.098	.033	.007	.019
OXSR1	Pearson Correlation	-.915*	-.907*	-.844*	-.852*	-.908*	-.955**	-.892*
	Sig. (2-tailed)	.011	.013	.034	.031	.012	.003	.017
Ceruplasmingene	Pearson Correlation	.957**	.943**	.963**	.977**	.842*	.930**	.967**
	Sig. (2-tailed)	.003	.005	.002	.001	.036	.007	.002
VDR	Pearson Correlation	.885*	.739	.749	.753	.915*	.737	.711
	Sig. (2-tailed)	.019	.093	.087	.084	.011	.095	.114
CAMK4	Pearson Correlation	.859*	.886*	.948**	.953**	.711	.915*	.922**
	Sig. (2-tailed)	.029	.019	.004	.003	.113	.010	.009

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

TABLE 5. serum antioxidant, bone markers and gene expression of ……

		SOD gene	CAT gene	GPX gene	OXSR1	Ceruplasmin gene	VDR gene	CAMK4 gene
Osteocalcin	Pearson Correlation	.880*	.921**	.836*	-.971**	.798	.829*	.789
	Sig. (2-tailed)	.021	.009	.038	.001	.057	.041	.062
Cp	Pearson Correlation	.938**	.840*	.953**	-.964**	.846*	.775	.813*
	Sig. (2-tailed)	.006	.036	.003	.002	.034	.070	.049
GPx	Pearson Correlation	.900*	.982**	.784	-.960**	.874*	.823*	.872*
	Sig. (2-tailed)	.015	.001	.065	.002	.023	.044	.023
SOD	Pearson Correlation	.970**	.885*	.928**	-.937**	.894*	.676	.930**
	Sig. (2-tailed)	.001	.019	.008	.006	.016	.140	.007
CAT	Pearson Correlation	.901*	.974**	.769	-.947**	.913*	.842*	.876*
	Sig. (2-tailed)	.014	.001	.074	.004	.011	.036	.022
TAC	Pearson Correlation	.777	.790	.755	-.871*	.820*	.927**	.645
	Sig. (2-tailed)	.069	.062	.082	.024	.046	.008	.167
MDA	Pearson Correlation	-.781	-.768	-.723	.772	-.947**	-.882*	-.746
	Sig. (2-tailed)	.067	.075	.104	.072	.004	.020	.089

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed) .
+ve mean positive correlation.-ve mean negative correlation

Fig. 1. show easily detached wool& alopecia on back.

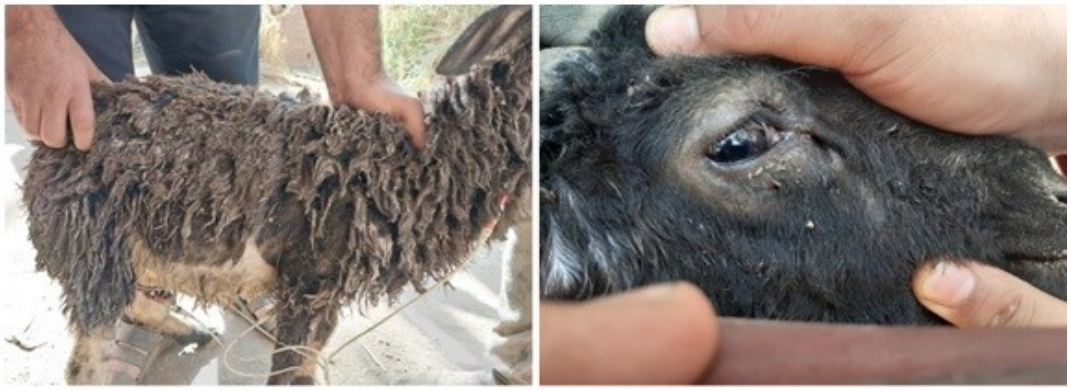

Fig. 2. 6 months male suffer from emaciation matted wool, change color of wool around eye and retardation of growth

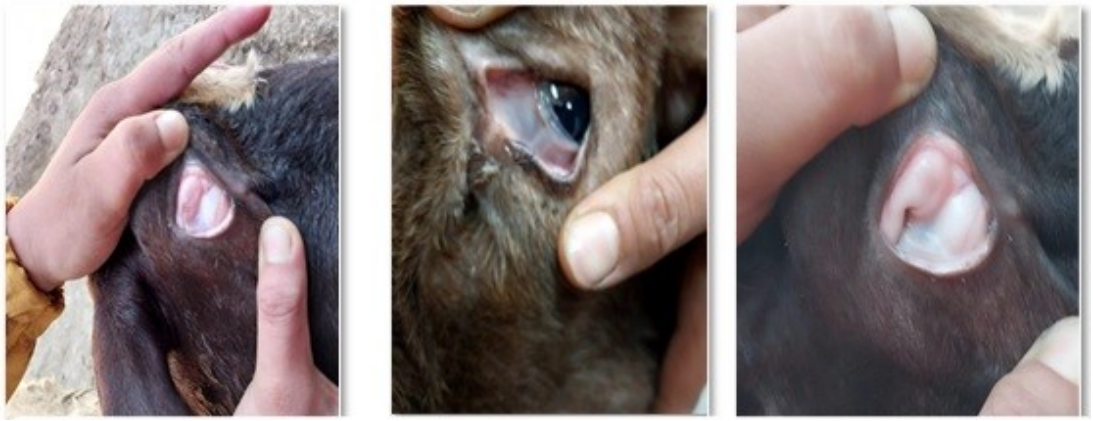

Fig. 3. show examination of conjuncitival pale Mucous membrane

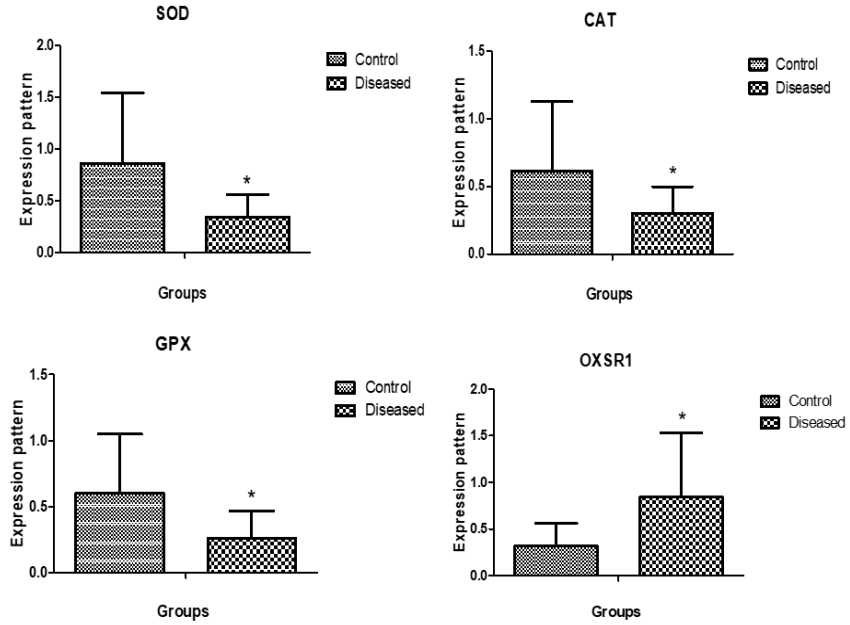

Fig. 4. mRNA levels of SOD, CAT, GPX, and OXSR1 genes in control and deficient sheep. Results are expressed as means ± SEM. *Values with asterisk were statistically significant (P<0.05).

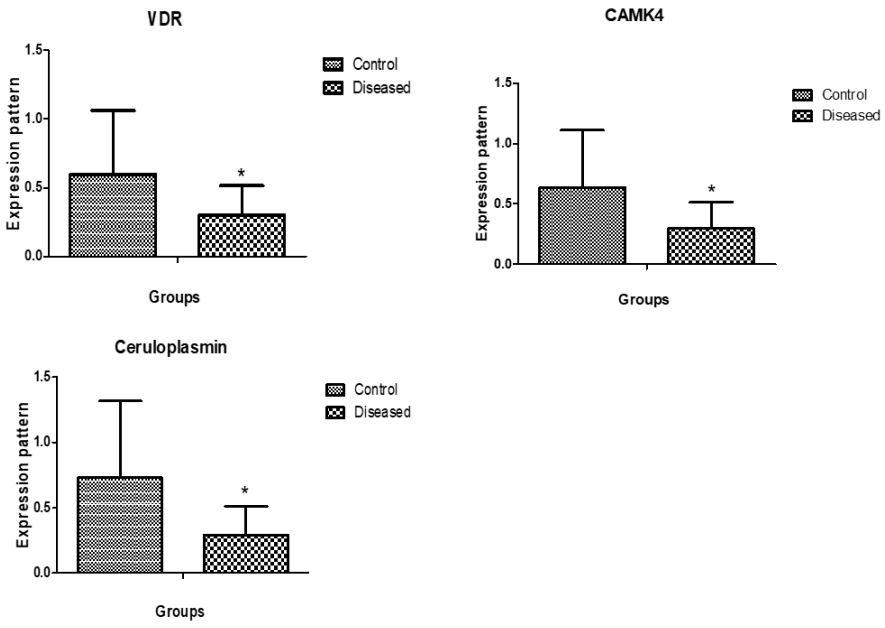

Fig.5. mRNA levels of VDR, CAMK4, and ceruloplasmin genes in control and deficient sheep. Results are expressed as means ± SEM. *Values with asterisk were statistically significant (P<0.05).

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التعبير الجيني لعلامات الأكسدة/مضادات الأكسدة، VDR، CAMK4 والسيرولوبلازمين في الأغنام البلدي التي تعاني من نقص المعادن

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الملخص

يهدف هذا العمل من هذا العمل هو تطوير التعبير الجيني والملف المصلي للأكسدة / مضادات الأكسدة وعلامات العظام المرتبطة بنقص المعادن في الأغنام. أجريت الدراسة على 26 حالة مرضية مختارة من المسح على 250 أغنام تعاني من نقص المعادن مقارنة بـ 10 أغنام ظاهريا صحية بناءً على الفحص الاكلينيكي. من الناحية الكيميائية الحيوية، كان هناك انخفاض معنوي (P <0.05) في الكالسيوم، الفسفور، الماغنسيوم، النحاس، الزنك،السيلينيوم، الحديد، النسبة الكليه لمضادات الاكسده ، GPx، SOD، CAT، OC وCP، في حين ارتفع مستوى MDA بشكل ملحوظ (P <0.05) . كان التعبير الجيني لجينات SOD وCAT وGPX1 وVDR وCAMK4 والسيرولوبلازمين منخفض بشكل ملحوظ (P <0.05) في الأغنام المريضة. وقد أثار الجين OXSR1 اتجاهًا معاكسًا. يمكن أن يكون التباين في الجينات المدروسة جنبًا إلى جنب مع التغيرات في المصل للعلامات التي تم فحصها دليلاً مرجعيًا للحد من نقص المعادن في الأغنام من خلال وصف استراتيجية فعالة لإدارة التغذية لقطعان الأغنام والتي تتضمن العناصر الأساسية. الجوانب الكلية والجزئية، وعدم الاعتماد فقط على الرعي كمصدر أساسي للعلف للحفاظ على صحة الأغنام وإنتاجها.

الكلمات الدالة: التعبير الجيني، العنصر الجزئي الكلي، علامات الأكسدة/مضادات الأكسدة، علامة العظام، الأغنام.