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Biochemistry

Changes in Hormones and Some Antioxidant Markers That Correlated to Zinc Deficiency and Affecting Wool Growth in Male Lambs

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ABSTRACT

This research aimed to investigate the correlation between the hormones and anti-oxidant changes as a biomarker related to zinc deficiency that affecting wool growth. Twenty male lambs were divided into 2 group, 1st group G1 (N=10) control healthy group, which received a total mixed ration (TMR). While 2nd group (N=10) zinc deficiency group, which received a concentrate ration low in zinc with high calcium levels for 3 months, after diet analysis it contains zinc 23.56 ppm and calcium 1.61% as feed dry matter basis. The clinical feature of induced zinc deficient male lambs revealed area of alopecia around eye and nose, in addition to poor wool growth with keratinization and crusts and scales formation on ear and may scattered all over the body. The result of group two serum analysis revealed significantly decrease anti-oxidants (SOD, CAT, GSH and TOAC) andhormones (Growth, thyroids and testosterone) while significantly increase in calcium and anti-oxidant of MDA with T4 hormone in comparison with group one, with a strong positive and reverse correlation to zinc level respectively. We concluded from our results that there was a correlation coefficient between the vital hormones and anti-oxidant changes as a biomarker related to zinc deficiency affecting wool growth in male lambs.

Key words: Anti-oxidants, Biomarkers, Hormones, Lamb and Zinc deficiency.

INTRODUCTION

Sheep considered the source of ovine meat and have an important value for their fleece and meat. Lamb's intestine has been formed into surgical sutures, similarly as strings for musical instruments and tennis rackets (William, 2006). While sheep play an important role in food production as a source of animal protein and constitute a vital source of wool which is considered the development of the economy for few countries (Hagos et al., 2018). The skin features a higher quantity of zinc mainly within the epidermis than within the dermis which is required for the proliferation and differentiation of epidermal cells (Hefnawy et al., 2018). The deficiency of zinc causes alopecia, crusting, scaling (McGavinand Zachary, 2007). Moreover, zinc incorporates a role in improving cellular integrity, growth of epidermal cells, keratin production, and epidermal keratinocytes proliferation and differentiation (Ogawa et al., 2016).

Zinc is considered an important trace element in animals (Wang et al., 2018) which plays a vital role within the structure and performance of the many macromolecules likewise as over 300 kinds of enzymes reactions. It plays a catalytic, structural and function role in enzymes (Gudrun et al., 2004). Zinc is one in all the key trace elements within the antioxidant

system. Moreover, zinc could be a vital component of the antioxidant system, which may prevent oxidation of cell membrane and reduce the super anions and cations formation. When zinc levels decreased, lipid oxidation increases, which might induce oxidative damage (Yousef and El-Hendy, 2002; Wen et al., 2018).Zinc deficiency resulted in an elevationof serum MDA content, and reduction in TOAC, CAT, GSH-Px, and SOD activity in sheep. Therefore, deficiency of zinc has serious affections on the function of antioxidant system thataffects growth and development, and by extension causes various diseases of animal body (Song and Shen, 2020).

Zinc has an important role on the reproductive functions besides in its effect on hormones (impacting on gonadotropins excretion) (Suchý et al., 1998). Zinc is closely associated with the male reproductive hormones therefore zinc deficiency in males causing hypogonadism (Prasad et al., 1996).Also, lack of zinc may affect the thyroid hormones production, additionally as testosterone levels (Yan et al., 2010). In zinc deficiency there was an elevation of T4 with reduction of T3 (Wada and King, 1986**)**. Zinc deficiency reduces the secretion of growth hormone from hypophysis and its circulating concentration (Roth and Kirchgessner, 1997). Low level of zinc in male lamb diet may lead to decrease in testosterone, free testosterone, TSH and T3 with increase in T4 levels (Tag El Din et al., 2022).

This research aimed to investigate the correlation between the hormones and anti-oxidant changes as a biomarker related to zinc deficiency that affecting wool growth.

MATERIALS AND METHOD

Experimental design:

This study was established within a private farm of sheep in Qalubia governorate. Twenty male lambs aged 10-12 months old were used. Lambs were classified into two groups, the first group as control healthy (N=10) and the second group for induced of zinc deficient group (N=10).

Zinc deficiency were induced by feeding of lambs on diet low in zinc with high calcium that contain corn soya bean meal, wheat bran, barley and rice straw, the calcium level was increased by addinga ground limestone 2% and steamed bone meal 2%, these for 3 months (Ibrahim et al., 2016). The control healthy group received total mixed ration (TMR) that contains concentrate diet, wheat and rice straw, alfa alfa hay and green roughage. Distilled water was offered *ad-libitum*. The analysis of concentrate diet of both group were recorded in table (1).

Table (1): The concentrate diet analysis of zinc deficient group and control healthy group:

Parameters	Concentrate diet of zinc deficient group	Concentrate diet of control healthy group
Protein (%)	14.1	16.14
Crude fiber (%)	32.4	35.21
Calcium (%)	1.61	0.77
Phosphorus (%)	0.67	0.48
Zinc (mg/kg DM)	23.56	33

Samples:

The samples of concentrate dietswere collected in a clean dry plastic bag and preserved in room temperature until examination.

The blood samples were collected from jugular vein (Pugh, 2002) from control

healthy and deficient groups. The blood samples were centrifuged at 3000 rpm for 15 minutes to obtain the serum. The clear sera were transferred into clean dry labeled Eppendorf tubes, and stored at 4°C for hormonal examination and −20°Ctill biochemical examination.

Concentratediet samples examination:

The collected concentrate diet samples were examined forprotein, crude fiber, calcium, phosphorus and zinc according to AOAC (2019).

Serum zinc analysis:

Zinc level was detected by atomic absorption Spectrophotometer according to method described by (Maret and Henkin, 1971) as following:

Instrument:

A National Institute for Standard (NIS) calibrated Atomic absorption spectrometer (Model SensAA-Dual and serial number A7942), which was manufactured by GBC Scientific and equipped with one optical channel Zinc is determined at 213.9 nm, the instrument is equipped with a 1-slot burner. All instrumental adjustments are made according to the manufacturer's instruction manual.

Reagents:

1. Water: Deionized distilled water was used throughout.
2. Diluent: Sixty milliliters of analytical grade n-butyl alcohol was diluted to 1 liter with water. The blank reading of this solution did not differ from zero when measured by AAS at the major absorption lines for zinc.
3. Sodium chloride solution: Analytical grade NaC1 was dissolved in water to make a final concentration of 1.5 mol / liter.
4. Standard solution: Zinc reference standard solution (Sigma Aldrish CRM made in Switzerland lot: BCCC2196 and Pcode: 102171465.) was used as stock solution. this solution contains 1 mg of zinc per ml., 10 ml of NaCl solution was added, and then diluted to 100 ml with water to produce standard solutions containing 10, 25, 50, 100 µg/100 ml of zinc in 150 millimolar NaC1.
5. Working standard solutions: One part of the solution was diluted with nine parts of diluent with a Fisher diluter, Model 250. To accomplish this, 4.5 ml of diluent and 0.5 ml of each standard solution were thoroughly mixed in a capped plastic vial these solutions were used to calibrate the instrument.
6. Unknown solution: Diluent, 4.5 ml, and 0.5 ml of each sample of serum were mixed as described for the working standard solution.

Estimation of Zinc:

Initially, while diluent was aspirated, the digital display of the A Channel was set atzero with the "auto zero" and "zero" controls. Then the zinc working standard (100 µg/100 ml) was aspirated, and the digital displays were set at 100. The other concentrations of working standards were then aspirated to verify the calibration accuracy and the linearity of instrument performance. After calibration, samples were aspirated and the results on the digital displays, in direct concentration units, were recorded manually. Diluent was aspirated betweensamples, and a working standard (100 g/100 ml) was aspirated after every 10 samples. If there were any deviations from zero or 100 on either digital display, the calibration controls were readjusted before further samples were aspirated. The concentration of zinc in aliquots of lamb serum was estimated with each set of samples.

Serum biochemical parameters examination:

Special kits were used for calcium (Ca) determination by methods described with Teitz (1986) and inorganic phosphorus (P) determination by method described by Young et al. (1975).

Special kits (Biodiagnostic Company) CAT. No. SD2521 was used for the

estimation of Superoxide dismutase (SOD) activity according to Nishikimi et al. (1972) and CAT. No. TA2513 was used for estimation of total antioxidant capacity (TOAC) according to Koracevic et al. (2001), reduced glutathione (GSH) level was determined by a method described by Paglia and Valentine (1967), catalase (CAT) activity was determined by a method described by Aebi (1984) and malondialdehyde (MDA) was determined calorimetrically according to the method recorded by Mesbah et al. (2004).

Quantitative determination of serum thyroid stimulating hormone (TSH); Triiodothyronine (T3) and thyroxin (T4) were determined by using ELISA kit (Immunospec corporation, USA, catalog No. E29-227, E29-229 and E29-230, respectively). The assay is based on a solid phase enzyme-linked immunosorbent assay with sensitivity 0.2µIU/ml, 0.25ng/ml and 0.5 µg/dl, respectively. Also, quantitative determination of serum testosterone, free testosterone and Growth hormone were determined by using ELISA kit (abia testosterone, catalog No. Dk 040 013; DBC, Canada, catalog No. CAN-FTE-260and GH ELISA kit, catalog No. MBS-743413 with sensitivity 0.2 ng/ml; 0.018 pg/ml and 0.1ng/ml, respectively.

Statistical Analysis:

The results were demonstrated as means ± SE. The data were statistically analyzed using SPSS 16 software to test correlation between zinc and therefore the other parameters. The differences were assessed by Student's t test of Excel Microsoft. The results were considered statistically significant when $P<0.05$, highly significant at $P<0.01$ and very highly significant at $P<0.001$.

RESULTS

Clinical signs:

The clinical signs recorded of induced zinc deficient group (Figure 1) revealed area of alopecia around eye, nose and tests, in addition to poor wool growth with keratinization, crusts and scales formation on ear and in the deferent parts of the body with roughness of wool.

Serum biochemical antioxidant markers:

The result of serum biochemical analysis (Table 2) of zinc deficient group revealed very highly significant ($P<0.001$) decrease in zinc level, while significantly ($P<0.05$) increase in calcium level and non-significant change in phosphorus. While calcium level revealed a reverse negative (-ve) correlation to zinc level.

The result of serum anti-oxidant examination (Table 2) of zinc deficient group revealed a very highly significant ($P<0.001$) decrease of SOD, CAT, GSH and TOAC with a highly strong positive correlation to zinc level, on contrary a significant ($P<0.05$) increase MDA with a weak reverse negative (-ve) correlation to zinc, these compared to control healthy group.

Serum hormones:

The result of serum hormones analysis (Table 3) of zinc deficient group revealed a very highly significant ($P<0.001$) decrease in growth hormone (GH), TSH, T3, T3/T4, testosterone and free testosterone with a highly strong positive correlation to zinc level, on the other hand there was very highly significant ($P<0.001$) increase in thyroxin (T4) hormone with a strong reverse negative (-ve) correlation to zinc level, these results compared to control healthy group.

Table (2): The result of serum biochemical and anti-oxidant analysis of zinc deficient group compared to control healthy group and correlation to zinc:

Groups / Parameters	Control group	Zinc deficient group	Correlation Coefficient (R)
Zinc (mg/dl)	**106.08 ±8.49**	**76.86 *** ±11.99**	**1**

Calcium (mg/dl)	**8.92 ±0.42**	**9.42 * ±0.35**	**-0.442 ***
Phosphorus (mg/dl)	**4.82 ±0.37**	**4.64 ±0.53**	**0.22**
SOD (U/ml)	**29.59 ±3.38**	**17.92 *** ±3.69**	**0.656 ****
CAT (U/ml)	**15.807 ±0.966**	**13.33 *** ±1.03**	**0.606 ****
GSH (U/ml)	**6.272 ±0.727**	**4.43 *** ±0.76**	**0.633 ****
TOAC (mU/L)	**1.737 ±0.068**	**1.545 *** ±0.078**	**0.619 ****
MDA (nmol/ml)	**1.555 ±0.104**	**1.71 * ±0.14**	**-0.174**

Data are expressed as mean ± SE of 10 samples. * Means significantly different at P<0.05 ** Means highly significantly different at P<0.01 *** Means very highly significantly different at P<0.001

(R) Close to the value of I indicate the strong positive correlation, (-ve) sign means reverse (negative) correlation.

Table (3): The result of serum hormones analysis of zinc deficient group compared to control healthy group and correlation to zinc:

Groups / Parameters	Control group	Zinc deficient group	Correlation Coefficient (R)
GH (ng/ml)	**1.36 ±0.16**	**0.63 *** ±0.23**	**0.629 ****
TSH (µU/L)	**0.56 ±0.08**	**0.415 *** ±0.079**	**0.56 ***
T3 (ng/ml)	**1.69 ±0.09**	**1.39 *** ±0.12**	**0.804 ****
T4 (µg/dl)	**49.39 ±2.61**	**64.26 *** ±4.48**	**-0.709 ****
T3/T4 (%)	**0.034 ±0.002**	**0.022 *** ±0.002**	**0.817 ****
Testosterone (ng/ml)	**5.14 ±0.53**	**3.412 *** ±0.614**	**0.564 ****
Free testosterone (pg/ml)	**0.464 ±0.038**	**0.248 *** ±0.058**	**0.784 ****

Data are expressed as mean ± SE of 10 samples.

* Means significantly different at P<0.05

** Means highly significantly different at P<0.01

*** Means very highly significantly different at P<0.001 (R) Close to the value of I indicate the strong positive correlation, (-ve) sign means reverse (negative) correlation.

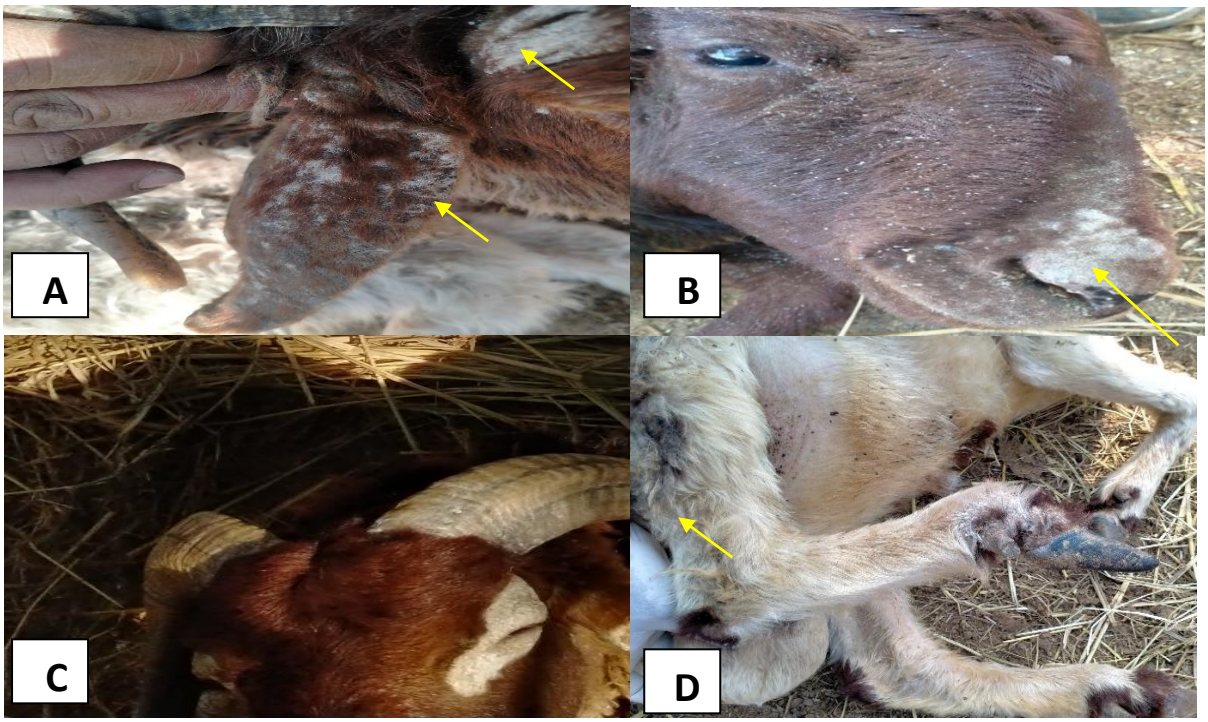

Figure (1): The clinical signs of zinc deficient groups. (A) Area of alopecia around eye and keratinization with crusts and scales formation on ear. (B) Alopecia and crusts formation around nose. (C) Alopecia around eye. (D) Roughness of wool and poor wool growth on tests and legs.

DISCUSSION

The signs of area of alopecia around eye, nose and tests, additionally to poor wool growth with keratinization, crusts and scales formation on ear and in the deferent parts of the body with roughness of wool within the zinc deficient lambs, agreed with the results recorded by (Masters et al., 1985; McGavin and Zachary, 2007; Song and Shen, 2020). These results of wool and skin abnormalities is also associated with the role of zinc in improving the cellular integrity, growth of epidermal cells and proliferation and differentiation of epidermal keratinocytes (Ogawa et al., 2016; Hefnawy et al., 2018).

The results of significantly decreased serum zinc level and increased calcium level related to the diet high in calcium and deficient in zinc these agreement with Ibrahim et al. (2016) who reported experimentally zinc deficiency signs appeared after feeding diet contains 26ppm zinc with high calcium level after 8-12 week. This might be attributed tothe higher level of calcium in diet that have adversely affect on zinc utilization (Mills et al., 1967; Krebs, 2000).

The results of serum antioxidants those significantly decreased SOD, CAT, GSH and TOAC with a significantly increased MDA, these in agreement with Song and Shen (2020). These results might be associated to the role of zinc as antioxidant activators and thought of one of amongst the key trace elements within the antioxidant system (Wen et al., 2018). During which zinc plays a significant role within the structure and performance of the many macromolecules moreover as over 300 forms of enzymes reactions. It plays a catalytic and structural role in enzymes (Gudrun et al., 2004). Moreover, zinc is a vital component of antioxidant system, which may prevent cell membrane oxidation and reduction of the super anions and cations formation. During zinc level

reduction, the lipid oxidation elevated, which can inducing the oxidative damage (Yousef and El-Hendy, 2002). Zinc considered an activator of glutathione peroxidase, which convert the glutathione into oxidized glutathione and decrease its content, a process that depends mainly on glutathione peroxidase, which help in protection of the body from oxidative damage through catalyzing the organic and inorganic peroxides reduction of the organism (Zhou et al., 2014). Within zinc absence, the lipid peroxide production increases and reduces the consumption of glutathione peroxidase, leading to reduction in the number of active glutathione peroxidase in the body (Li et al., 2001). Deficiency of zinc leading to reduction of SOD and glutathione peroxidase activity, which results in impairment of the removal of superoxide anion free radicals, induction of the peroxidation of tissue lipid, and resulting in elevation of malondialdehyde content of lipid peroxidation product, that reducing the body's ability to remove free radicals seriously affects the function of antioxidant system within the body (Cao and Lu, 2010).

The significantly decreased growth hormone (GH), TSH, T3, T3/T4, testosterone and free testosterone and significantly increased T4 hormone, these in agreement with Tag El Din et al. (2022) who reported that in male lambs suffered from some trace elements deficiency mainly zinc resulted in decreased growth and sexual hormones and increased T4 levels. The decrease in growth hormone (GH) level, may be associated to zinc deficiency which reduces the secretion of somatotropin from hypophysis and its circulating concentration (Roth and Kirchgessner, 1997). Moreover, zinc has a role in combination between growth hormone receptors and its growth factor binding protein-3 (IGFBP-3) so that zinc deficiency resulted in decreases this combination (McNall et al., 1995). Zinc deficiency leading to decrease levels of thyroid hormones secretion further as testosterone levels (Yan et al., 2010). The decreased level of T3 with elevated T4 may be associated to the activity of type I-5'deiodinase enzyme and consequently conversion of T4 to T3 is reduced in zinc deficiency (Wada and King, 1986). Zinc could also be necessary for the enzymes involved in TSH synthesis (Pekary et al., 1991). The decreased testosterone and free testosterone hormones could also be associated with zinc deficiency. Thus, zinc is closely associated with the male reproductive hormones. Deficiency of zinc in males causes hypogonadism (Prasad et al., 1996).

The results of correlation between zinc level and therefore the other parameters providing that the changes in hormones and antioxidants accompanied by wool and skin abnormalities can be considered as biomarkers of zinc deficiency in male lambs. Plasma zinc is also a useful biomarker for risk of spontaneous miscarriage (Graham et al., 1995).

CONCLUSION

We can concluded that; zinc is an essential trace element in lambs, it is involved in the synthesis of many enzymes which plays a vital roles in the animal antioxidant system. Zinc deficiency may lead to changes in the level of vital hormones, antioxidants, wool, and skin abnormalities. There was a correlation between zinc level and changes in hormonal level and antioxidants which could be considered a biomarker for zinc deficiency in male lambs which can help veterinarians and flock owners in diagnosis of zinc deficiency subclinical cases in our Egyptian lamb's farms.

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