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### Some hormonal changes related to some trace elements deficiency in ration of male lambs

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#### ABSTRACT

**T**race element deficiency in male lambs is a serious problem. It refers to either insufficient nutrients or mineral elements imbalance. This work aimed to investigate the hormonal and some biochemical changes related to some trace element deficiency and the effect of mineral mixture supplementation on these changes. To achieve this aim we induced trace element deficiency in a tested male lambs group (N=10) belonged to our sheep herd and received a mineral mixture as a feed supplement for 10 days. The analysis of tested diet revealed protein 12.32%, calcium 1.21%, phosphorus 0.27%, and zinc 25.96 mg/kg DM. While the control healthy group (N=10) received a traditional diet. The results of serum hormones and biochemical analysis of tested group revealed a significant ( $P<0.05$ ) decrease in TSH, T3, T3/T4, testosterone, free testosterone, SHBG, GH, albumin and zinc, with a significant ( $P<0.05$ ) increase in T4, total protein and globulin compared to control healthy group. On the other hand, the results obtained after mineral mixture supplementation revealed a significant ( $P<0.05$ ) increase of TSH, T3, T3/T4, testosterone, free testosterone, SHBG, GH, albumin and zinc. Also, a significant ( $P<0.05$ ) decrease in T4, total protein, and globulin was observed compared with this group before mineral mixture supplementation, with observation of the changes were gradually toward recovery. Moreover, insignificant changes were recorded in calcium, phosphorus and iodine. In conclusion to trace element deficiency have an effect on vital hormones with extension to metabolic process, general health condition and proper growth of male lambs.

#### INTRODUCTION

Trace elements are important for raising lambs. So a new fed ingredients could be used in lamb diet that will be contributed to increase weight gains and improvement of meat quality (Valença *et al.* 2020). However, the goal of productive efficiency in feedlot animals, relies

upon many factors, including the strategy of how diet is fed (De-Oliveira *et al.* 2020). Increasing the frequency of feeding diets composed of hay and concentrates could stimulate the appetite of feedlot and productivity (Saldanha *et al.* 2021). The proper growth and improvement of growing lambs mainly rely

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upon the level of nutrition, on contrary insufficient nutrition may decrease the growth performance of intensively managed sheep (Ebrahimi *et al.* 2007).

Mineral elements are nutritionally essential for small ruminants. These include macro-minerals as calcium, phosphorous, sodium, chlorine, potassium, magnesium, and sulfur. Trace elements, or micro minerals as copper, selenium, zinc, iodine, cobalt, iron, manganese and molybdenum. Improvement of trace element nutrition that meet consumer perceptions and preferences, is a current cost effective challenge (Radostitis *et al.* 2007).

Zinc (Zn) is an important microminerals essential for all animals (Suttle 2010). The counseled minimal necessities of zinc are 20 mg/kg DM for growth and 33 mg/kg DM for maintenance of normal reproductive function including male testicular development and female pregnancy and lactation. The high calcium diet (about 1.2-1.8% calcium) adversely affect zinc utilization (Mills *et al.* 1967). The effect of zinc on reproductive functions besides its effect on hormones (impacting on gonadotropins excretion, influencing the sperm motility and their ability to penetrate the ova) (Noakes *et al.* 2001). Zinc deficiency results in decreased levels of thyroid hormones secretion which affecting the normal feed metabolism and resting metabolic rate. This low level also badly affects testosterone levels (Yan *et al.* 2010).

Iodine is necessary for thyroid hormone synthesis with normal basal metabolic rate (BMR) and reproductive energy metabolism (Suttle 2010). Iodine is essential for thyroxin synthesis with general metabolic regulation, particularly mitochondrial activity. Decreased thyroxin is associated with non-specific signs of poor growth, loss of libido, hence affects the animal reproductive performance (Sargison *et al.* 2011).

Calcium (Ca) and phosphorus (P) are closely interrelated, particularly in the development and maintenance of skeletal system function. The high calcium diet may cause a deficiency of another elements, as phosphorus, magnesium, iron, iodine, zinc, and manganese (NRC 2007).

Blood thyroid hormone level indicates the changes in thyroid gland activity and circulat-

ing thyroid hormones that reflects the metabolic and nutritional status of the animal (Todini 2007; Antunović *et al.* 2019).

Additional supplementations are generally required because the native forages often not fulfills the growth requirements (Ward & Gifford 2017). Therefore mineral mixtures are usually mixed with concentrates diet for improving the growth rate, reproduction, feed utilization, immune response and general health (Kalita *et al.* 2003). The supplementation of mineral mixture in conventional adequate diets of lambs were helpful in improving the daily body weight gain, feed efficiency and feed conversion ratio (Kharb *et al.* 2017).

The aim of this work is to investigate the effects of nutritional trace elements deficiency on some hormones and serum biochemical with the role of mineral mixture supplementation in recovery of the changes occurred.

## MATERIALS AND METHODS

### Experimental design

The study were established in a sheep farm in Kalubia governorate. Ten male lambs aged 9 -12 months were isolated received diet composed of imbalanced trace elements and examined before and after mineral mixture supplementation. The animals were compared to control healthy group (N=10) that feeding by traditional methods (using roughage, corn grains, wheat bran, barely, concentrates, wheat straw, rice straw).

### Supplementation

Mineral mixture, composed of a group of minerals zinc, manganese, iron, copper, iodine, selenium, cobalt, sodium chloride, phosphorus and calcium carbonates manufactured by AGRIVET Company, Egypt. This mineral mixture added to concentrated diet as feed additives according to the manufactured company (3 kg for one ton concentrate) for 10 days.

### Samples

The sample of diets were collected from the concentrate diet of the animals in a clean dry plastic bag and preserved at room temperature until examination.

The blood sampling were collected from the jugular vein (Pugh 2002) from control and at the time of disease detection and after the end

of 10 days of mineral mixture supplementation in the deficient group. The blood samples were centrifuged at 5000 rpm for 5 minutes to obtain the serum samples. The clear sera were aspirated carefully by automatic pipette and transferred into clean dry labeled Eppendorf tubes, and stored at  $-20^{\circ}\text{C}$  till examination.

## Methods

### Concentrated diet examination

The collected concentrated diet samples were examined for protein, fat, carbohydrates, TDN, crude fiber, calcium, phosphorus, zinc, iron, copper, magnesium, sodium, potassium and iodine according to **AOAC (2005)**. Also aflatoxin and ocratoxin were examined.

### Serum examination

#### Biochemical determination

Special kits (Stain Bio Company) were used for spectrophotometric determination of total protein, albumin, and a special kits (Biodiagnostic Company) were used for spectrophotometric determination of zinc, calcium, inorganic phosphorus and iodine.

#### Hormonal Determination

Quantitative determination of serum thyroid stimulating hormone (TSH); Triiodothyronine (T3) and thyroxin (T4) were determined by using enzyme immunoassay 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  $0.2\mu\text{IU/ml}$ ;  $0.25\text{ng/ml}$  and  $0.5\mu\text{g/dl}$  for TSH, T3 and T4 respectively. Also, quantitative determination of serum testosterone, free testosterone, sex hormone binding globulin (SHBG) and growth hormone were determined by using enzyme immunoassay ELISA kit (Abia testosterone, catalog No. Dk 040 013; DBC, Canada, catalog No. CAN-FTE-260; Cloud-Clone Crop., catalog No. E-0095BO and catalog No. MBS-743413 with sensitivity  $0.2\text{ ng/ml}$ ;  $0.018\text{ pg/ml}$ ;  $1\text{ ng/ml}$  and  $0.1\text{ ng/ml}$  respectively.

#### Statistical analysis

The data were statistically analyzed using one way (ANOVA) as previously described (**Bailey 2008**) using SPSS 16 software to test the significance difference between control group and the diseased group before and after

mineral mixture supplementation. The results were demonstrated as means  $\pm$  SE. The results were considered statistically significant when  $P<0.05$ .

## RESULTS

The clinical examination of diseased animal noted that the animals were weakened, depressed with decreased appetite and weight loss. The result of feed analysis revealed negative (-ve) result of aflatoxin and ocratoxin, protein 12.32%, TDN 69%, fat 1.51%, fiber 30.38%, calcium 1.21%, phosphorus 0.27% and zinc 25.96 mg/kg dry matter basis (DM). The other nutrients and minerals examined were within the reference range of nutrient requirement.

The results of serum biochemical examination of diseased animals before mineral supplementation (**Tab. 1**) revealed a significant ( $P<0.05$ ) increase in total protein, globulin with significant ( $P<0.05$ ) decrease in albumin level compared to control healthy group. On the other hand, the serum biochemical examination after mineral supplementation revealed a significant ( $P<0.05$ ) decrease in total protein and globulin with significant ( $P<0.05$ ) increase in albumin compared to this group before mineral supplementation. The serum minerals examination (**Tab. 1**) in diseased group before mineral supplementation revealed significant ( $P<0.05$ ) decrease in zinc level, but non-significant changes after mineral supplementation compared to control healthy group. Calcium, inorganic phosphorus and iodine revealed non-significant changes between groups.

The results of serum hormones examination (**Tab. 2**) revealed significant ( $P<0.05$ ) decrease in TSH, T3, T3/T4, testosterone, free testosterone, SHBG and GH with a significant ( $P<0.05$ ) increase in T4 of the animals suffered from nutritional trace element deficiency before mineral mixture supplementation compared to control healthy group. On the other hand, the results after mineral mixture supplementation revealed significant ( $P<0.05$ ) increase in TSH, T3, T3/T4, testosterone, free testosterone, SHBG and GH with a significant ( $P<0.05$ ) decrease in T4 in comparing to this group before mineral mixture supplementation and these changes were nearly toward the control level.

**Table (1):** Results of serum biochemical and minerals of the trace element deficient group before and after mineral mixture supplementation compared to control healthy group.

Parameters	Control group	Trace element deficient group	
		Before supplementation	After supplementation
<b>Total Protein (g/dl)</b>	7.045 ± 0.357 <sup>b</sup>	8.092 ± 0.473 <sup>a</sup>	7.275 ± 0.298 <sup>b</sup>
<b>Albumin (g/dl)</b>	3.442 ± 0.151 <sup>a</sup>	3.041 ± 0.17 <sup>c</sup>	3.265 ± 0.168 <sup>b</sup>
<b>Globulin (g/dl)</b>	3.603 ± 0.234 <sup>c</sup>	5.051 ± 0.332 <sup>a</sup>	4.01 ± 0.22 <sup>b</sup>
<b>Zinc (µg/dl)</b>	100.08 ± 5.07 <sup>a</sup>	80.86 ± 5.34 <sup>b</sup>	97.94 ± 5.9 <sup>a</sup>
<b>Calcium (mg/dl)</b>	9.12 ± 0.33	9.348 ± 0.416	9.284 ± 0.334
<b>Inorganic Phosphorus (mg/dl)</b>	5.218 ± 0.296	5.14 ± 0.55	5.193 ± 0.308
<b>Iodine (µg/dl)</b>	81.24 ± 7.31	82.31 ± 7.62	81.82 ± 6.22

Data are expressed as mean ± SE of 10 samples.

<sup>a, b, c</sup> Means within the same raw with different superscripts are significantly different at ( $P < 0.05$ ).

**Table (2):** Results of serum hormonal changes of the trace element deficient group before and after mineral mixture supplementation compared to control healthy group.

Parameters	Control group	Trace element deficient group	
		Before supplementation	After supplementation
<b>TSH (µIU/ml)</b>	0.43 ± 0.052 <sup>a</sup>	0.312 ± 0.054 <sup>b</sup>	0.384 ± 0.051 <sup>a</sup>
<b>Total T3 (ng/ml)</b>	1.817 ± 0.0696 <sup>a</sup>	1.49 ± 0.116 <sup>c</sup>	1.658 ± 0.055 <sup>b</sup>
<b>Total T4 (µg/dl)</b>	54.49 ± 0.644 <sup>c</sup>	69.86 ± 1.37 <sup>a</sup>	60.8 ± 0.96 <sup>b</sup>
<b>T3/T4 (%)</b>	0.033 ± 0.0012 <sup>a</sup>	0.021 ± 0.002 <sup>c</sup>	0.027 ± 0.0012 <sup>b</sup>
<b>Testosterone (ng/ml)</b>	4.429 ± 0.428 <sup>a</sup>	2.512 ± 0.556 <sup>b</sup>	4.503 ± 0.598 <sup>a</sup>
<b>Free Testosterone (pg/ml)</b>	0.333 ± 0.054 <sup>a</sup>	0.158 ± 0.071 <sup>b</sup>	0.288 ± 0.046 <sup>a</sup>
<b>SHBG (ng/ml)</b>	1630.8 ± 75.9 <sup>a</sup>	1406.4 ± 98.1 <sup>b</sup>	1616.8 ± 75.4 <sup>a</sup>
<b>Growth hormone (ng/ml)</b>	1.18 ± 0.047 <sup>a</sup>	0.126 ± 0.082 <sup>c</sup>	1.11 ± 0.071 <sup>b</sup>

Data are expressed as mean ± SE of 10 samples.

<sup>a, b, c</sup> Means within the same raw with different superscripts are significantly different at ( $P < 0.05$ ).

## DISCUSSION

The clinical examination of diseased animal showed that there were weakness, depression, decreased appetite and weight loss. These signs were in agreement with **NRC (2007)**. Malnutrition has a prominent effect on the production and reproductive functions (**Abecia et al. 2006**). The result of feed analysis revealed calcium 1.21%, phosphorus 0.27%, zinc 25.96 mg/kg DM, the other nutrients and minerals within the reference range of nutrient requirements. While, the daily nutrient requirement are calcium 0.42% and phosphorus 0.21% according to **NRC (2007)**. The nutrient requirements of zinc were 33 mg/kg DM needed for testicular maturation and normal reproductive function in male lambs (**Mills et al. 1967**). These results provided imbalance in minerals and trace element as the diet is high in calcium and low in zinc.

The serum minerals in diseased animals revealed decreased zinc level. This result agreed with **Ibrahim et al. (2016)** who reported experimental zinc deficiency after feeding diet contains 26ppm zinc with high calcium level after the 6th week. This also may be attributed to the elevated level of calcium in diet that adversely affects zinc utilization (**Mills et al. 1967**).

The analysis of serum biochemistry in diseased animals showed elevated total protein and globulin with decreased albumin. An increase in serum globulin indicates the development of an antibody response to the present antigen and deprived diet stress factors with nutritional zinc deficiency. Furthermore, increase the total protein is related to increased globulin (**Van den Broek et al. 2000**). On the other hand decreased albumin may be attributed to decreased appetite and the distorted hepatic protein metabolism. Loss of appetite and subsequently weight losses may develop changes in hepatic function and decrease in nutrients digestion (**Fisher & Crookshank 1982**).

The result of serum hormones of diseased animals revealed increased T4 and decrease in T3 and TSH. These results may be related to the nutritional zinc deficiency as a result of oxidative stress that leading to thyroid dys-

function (**Ganapathy & Volpe 1999**). Zinc deficiency in the body may result in decreased levels of secretion of thyroid hormones (**Yan et al. 2010**). The activity of type I-5' deiodinase enzyme and consequently the conversion of T4 to T3 is curbed in nutritional zinc deficiency (**Wada & King 1986**). Both the hypothalamus and hypophysis are susceptible to nutritional zinc deficiency. Consequently, it was suggested that zinc might be necessary for the enzymes involved in thyroid releasing hormone (TRH) and TSH synthesis (**Pekary et al. 1991**). Moreover, **Tag El-Din et al. (2015)** found that decrease in T3 levels in ewes may be due to zinc deficiency recorded in these animals as it has been reported that zinc participate in synthesis of protein and essential for thyroid function since involved in T3 binding to its nuclear receptor (**Liu et al. 2001**).

The decreased testosterone, free testosterone and sex hormone binding globulin (SHBG) in diseased animals may be related to nutritional zinc deficiency. Thus, Zn is closely related to the male reproductive hormones. Severe and moderate zinc deficiency in males causes hypogonadism (**Prasad et al. 1996**). Low level of zinc may affect the production of these hormones, as well as testosterone level (**Yan et al. 2010**). Zinc deficiency disrupts the activity of angiotensin converting enzyme, which is involved in the production of adrenal androgens (**Kwok et al. 2010**). This disruption results in decreased testosterone production and the inhibition of spermatogenesis (**Bedwal & Bahuguna 1994**). Nutritional zinc deficiency in male rat considerably inhibits testosterone (**Ozturk et al. 2005**). Also, deficiency of zinc suppresses the receptor activity of androgenic hormones and thus zinc has a critical part in the regulation of male reproductive functions (**Om & Chung 1996**). These results investigate the relation between nutrition trace element mainly zinc and hormones. Changes in serum hormones are indicator for nutritional status, animal healthy and reproduction status (**Todini 2007; Antunović et al. 2019**).

Drop in level of growth hormone (GH) may be related to nutritional trace element deficiency mainly zinc deficiency (**Roth & Kirchgess-**

ner 1997). Moreover, zinc deficiency resulted in decrease the affinity of growth hormone receptors and growth factor binding protein-3 (IGFBP-3) in rat (McNall *et al.* 1995).

The serum analysis of animals after mineral mixture supplementation revealed an increased level of testosterone, free testosterone, SHBG, TSH, T3, T3/T4, GH and zinc with decreased T4 level in comparison with this group before mineral supplementation. These results provided the role of mineral mixture supplementation in compensation of zinc and leading to the recovery of vital and reproductive hormones. Supplementation of mineral mixture in conventional lamb diets is helpful in improving the daily body weight gain, feed efficiency and conversion ratio, production and reproduction performance (Kalita *et al.* 2003; Kharb *et al.* 2017).

## CONCLUSION

Based upon the results of this work it could be concluded that; Nutritional trace elements deficiencies mainly zinc in male lambs is a serious problem. It refers to either insufficient or imbalance between mineral and trace elements levels. The main nutritional trace element deficiency in this work was zinc deficiency that occurred due to high calcium diet. Nutritional zinc deficiency leading to changes in thyroid hormones, growth hormone and sexual hormones not only but also some serum biochemical. Mineral mixture supplementation in conventional lamb diet was helpful in improvement of reproductive hormones and for compensation of mineral requirements.

## Recommendation

Nutritional trace elements requirement are very necessary to be concerned by using total mixed ration (TMR) either during growing and fattening or reproduction of lambs. Also using a traditional method of feeding is satisfy.

Supplementation of mineral mixture in the lamb diets is recommended and could be used all over the period of growing, fattening and reproduction.

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