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Effect of Serum Trace Minerals on the Metabolism of Thyroid Hormones in Male and Female Lambs

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Abstract: Based on genetic factors, trace minerals are essential for thyroid function and hormone metabolism. For this purpose, serum levels of thyroid hormones and some blood micronutrient concentrations in lambs were determined, compared and their relationships were investigated. The 176 lambs including 70 males under 2 months old and 106 females aged 2-8 months were selected from Ghezel and Makui breeds. The 5 mL Jugular blood was prepared and the sera were evaluated for the concentrations of T3, T4, TSH, copper (Cu), iron and zinc. Thyroid hormones were determined by ELISA and trace minerals by autoanalyzer method. Mean T3 and TSH in males were higher than in females and T4 in females were higher than in males but only TSH was significant ($p<0.01$). The mean serum concentrations of Cu, iron (Fe) and zinc (Zn) in males were lower than in females in that only Cu and Zn were significant ($p<0.01$) and iron was not different. TSH in males was 36.3% higher than in females, Cu and Zn in females were 12.6% and 7.3% higher than in males, respectively. The T3:T4 ratio was higher in males than in females and for overall samples the T3:T4 ratio was low. There were significant negative relationships between T3/TSH ($r = -0.36$) and T4/Cu ($r = -0.24$) in males but not in females. There was a significant negative relationship between TSH/T3 ($r = -0.16$) for all lambs. Significant positive relationships were found between Cu/Zn ($r = 0.38$) and Fe/Zn ($r = 0.44$) in males, females and for overall ($p<0.01$).

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INTRODUCTION

Copper (Cu) is considered as a trace element[1] and is an essential cofactor for a range of enzymatic reactions[2]. It is essential for the function of other nutrients including Fe[3]. Copper as a component of metalloenzyme, plays a role in tissue oxidation by complementing the cytochrome oxidase system[4]. Copper enzymes include ceruloplasmin, cytochrome oxidase, superoxide dismutase, tyrosinase, lysyl oxidase, dopamine beta-hydroxidase and monoamine oxidase[2]. It is absorbed in the intestine and then bound to sulfhydryl groups such as albumin and metallothionein to be transported to the liver for storage, so, it is necessary in growth, production and reproduction. Its amount in the ewe's blood must be sufficient for the growth and development of the fetus, otherwise reproductive, congenital and neurological disorders will be occurred[5]. The most prominent symptoms of Cu deficiency in lambs are enzootic ataxia and cerebral-spinal disorders[6], so, Cu supplements eliminate the deficiency soon[7, 8]. It has variable relationships with organic, inorganic elements and thyroid hormones as well. It has a positive relationship with Fe and a negative correlation with Zn[9]. In one study, Cu-deficient ewes had low levels of Cu and T4 but normal T3, meaning that there was a positive relationship between Cu and T4, so, Cu deficiency is associated with T4 deficient[10]. Pop *et al*.[3] and Jain *et al*.[1] reported a positive correlation between Cu and thyroid hrmones in women. Finally, Fe and Cu are vital for the synthesis of thyroid hormones[11], so, Cu evaluation is important in determining the status of thyroid hormones.

Zinc as a metalloenzyme thymidine kinase is an essential micronutrient in growth, production and reproduction[2]. It is important for energy production through carbohydrate metabolism, protein synthesis for growth and reproduction, enhancing the immune system and cell membrane resistance to microorganism as an antioxidant[12]. Zinc is involved in regulating diodinase activity, thyroid-releasing hormone and TSH (thyrotrophin) synthesis[3]. Zinc acts as an inhibitor or cofactor of diodinase enzymes[13]. It also acts as a cofactor for dehydration reactions that convert T4 to T3[14]. Zinc deficiency causes thyroid atrophy, decreased T3 and T4 and systemic effects[15]. Zinc consumption increases up to 50% in pregnant ewes due to fetus growth, so, its decrease is high in heavy pregnancies and in lambs as well[16].

Zinc in forage is hardly hydrolyzed by enzymes in the intestines, so, Zn deficiency will occur in sheep in lack of Zn supplementation[12]. Zinc deficiency is accompanied by increased hematocrit, progesterone changes, decreased immune competition, abnormal platelets and bleeding and finally, fetal growth retardation[16].

Thyroid hormones including triiodothyronine (T3) and thyroxine (T4) affect the predominant activity of body cells and their role is considered important in terms of cell function and body systems[17]. Thyroid hormones are involved in regulating energy consumption, RNA synthesis, cell oxygen consumption, the body's overall metabolism and the growth and development of the nervous system. Therefore, the major role of T3 and T4 is vital in maintaining growth and especially milk production, so, determining their values will lead the metabolism and nutritional status in animals[18].

Decreased T3 leads to stimulation of the hypothalamic-pituitary axis to secrete Thyroid Stimulating Hormone (TSH)[13] which is also one of the evaluation criteria for thyroid hormones. Iodine is a key in the structure of these hormones and its deficiency (hypothyroidism) in ewes is associated with poor wool growth, milk and weight loss, reproductive system disorder[19], decreased sexual activity of rams, abortion and weak births with signs of goiter and increased susceptibility to infections[20] which are also seen in Cu and Zn deficiencies.

The results of genetic factors show that trace minerals such as Cu and Zn, etc. are essential for thyroid activity and hormone metabolism[11] as well as some metals have pathogenic effects on the thyroid[21].

Since, Cu and Zn deficiencies have similar signs to hypothyroid hormone depletion in lambs[22] and their deficiencies is highly probable in lambs, it is necessary to extract their values and interactions among micronutrients and thyroid hormones. The results could improve the breeding of small ruminants. The objectives were: To determine and comparison of the serum amounts of T3, T4, TSH and their ratio in male and female lambs. To determine serum concentrations of Cu, Fe and Zn between genders and to determine the relationships among thyroid hormones and micronutrient indices in lambs.

MATERIALS AND METHODS

Animals: The number of 176 lambs including 70 males under 2 months old and 106 females aged between 2 and 8 months were selected from Ghezel and Makui sheep herds. Sheep had free grazing on pasture consisted of legume and cereal forage without concentrate feeding. The suckling male lambs received milk from ewes twice in the morning and evening. Lambs were clinically healthy and did not have a disease problem. The 5 mL jugular blood was prepared by syringe from animals and were transferred to the test tube. After collection, samples were centrifuged at 3000 rpm for 10 min to separate the sera and then were frozen in -20°C for laboratory evaluations.

Tyroid hormones evaluation: Tyroid hormones including T3, T4 and TSH were evaluated by ELISA (Monobind, USA) based on competitive hormone

Table 1: Concentrations of thyroid hormones (nmol/L) and trace minerals (mg/dL) in males, females and total of lamb's sera

Parameters	Mean	SD	SE	Minimum	Maximum
Male lambs (n = 70)					
T3	0.842	0.03	0.26	0.14	1.85
T4	1.08	0.02	0.20	0.57	1.48
TSH	0.204	0.02	0.16	0.05	0.96
Copper	172. 2	4.3	35.9	89.1	290.0
Iron	86.81	1.61	13.4	58.8	142.0
Zinc	115.4	1.81	15.12	89.4	154.0
Female lambs (n = 106)					
T3	0.79	0.03	0.23	0.22	1.48
T4	1.19	0.06	0.57	0.21	2.57
TSH	0.13	0.01	0.08	0.01	0.56
Copper	197.1	5.34	54.9	83.1	460.0
Iron	90.5	2.53	26.1	35.7	239.0
Zinc	137.7	3.45	35.6	30.2	228.0
Total lambs (n = 176)					
T3	0.81	0.02	24	0.14	1.85
T4	1.15	0.04	0.46	0.21	2.75
TSH	0.16	0.01	0.13	0.01	0.96
Copper	187.1	3.75	49.7	83.1	460.0
Iron	89.1	1.65	21.9	35.7	239.0
Zinc	127.6	2.32	30.8	30.2	228.0

Table 2: Mean comparison of T3:T4 ratio (nmol/L) between male and female lambs

Parameters	Male lambs	Female lambs	Total lambs
T3:t4 Ratio	0.8	0.66	0.71
T3:TSH " " " "	4.13	6.1	5.1
T4: TSH" " " "	5.3	9.2	7.2

response and hormone-enzyme conjugation to bind to a limited number of anti-hormone monoclonal antibodies. The rate of light absorption of standard samples and sera tested was determined using ELISA Reader and calibration curve in terms of nmmol/l.

Trace mineral evaluation: The concentrations of Cu, Fe and Zn were determined by autoanalyzer (Technicon RA-1000, USA) using the relevant commercial kits of Pars Azmoun Iran in specific wavelengths. Results were determined in mg/dL.

Statistical analysis method: SPSS23 software program was appropriate to analyse the data. At first, data were tested to their normal distribution using Colmograph and then case summaries were used to determine mean, standard deviation, standard error and range of serum thyroid and micronutrient values. T-test was used to compare the mean of serum thyroid indices and trace minerals in male and female lambs. Chi-square test was used to determine the significant difference between the ratios of thyroid hormones (T3:T4) in male and female lambs. A value of $p<0.05$ was considered as a significant difference. Pearson correlation test was performed to determine the relationship between serum thyroid indices and micronutrients separately in each group of male and female lambs and then in overall data. The results were plotted and interpreted as Table 1-3 and Fig. 1-3 for each group of thyroid and trace mineral indices.

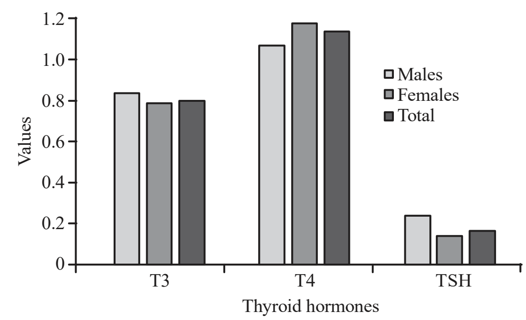

Fig. 1: Comparison of thyroid hormones concentration (nmol/L) in males, females and total lamb's sera

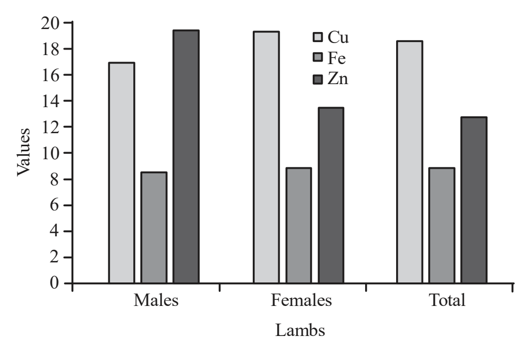

Fig. 2: Comparison of trace minerals concentration (mmol/L) in males, females and total lamb's sera

Table 3: Correlations among thyroid hormones and trace minerals in males, females and total of lamb's sera

Parameters	T4	TSH	Copper	Iron	Zinc
Male lambs (n = 70)					
T3	0.09	-0.36**	-0.11	0.03	0.05
T4		0.15	-0.24*	0.12	-0.19
TSH			0.04	-0.1	-0.15
Copper				-0.06	0.38**
Iron					0.44**
Female lambs (n = 106)					
T3	-0.09	-0.03	0.09	-0.01	-0.07
T4		-0.13	-0.11	-0.03	-0.1
TSH			0.14	-0.16	-0.12
Copper				0.04	0.22*
Iron					0.74**
Total lambs (n = 176)					
T3	-0.06	-0.16*	-0.01	-0.02	0.03
T4		-0.06	-0.07	-0.04	-0.06
TSH			-0.12	-0.13	-0.19*
Copper				0.04	0.30*
Iron					0.69**

** = p<0.01; ** = p<0.05

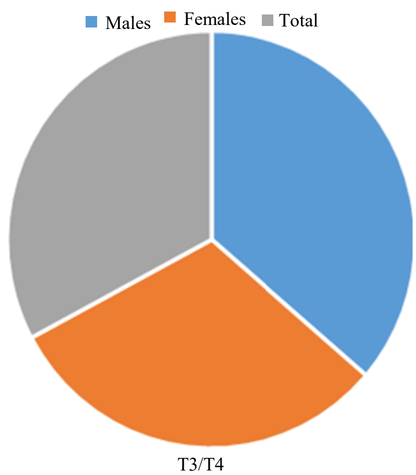

Fig. 3: Comparison of T3:T4 ratio (nmol/L) in males, females and total lamb's sera

RESULTS

The concentrations of thyroid hormones and trace minerals in males, females and overall was shown in Table 1. Mean T3 and TSH of males were higher than in females and T4 of females were higher than in males but only TSH was significant between gender (p<0.01). The mean serum concentrations of Cu, Fe and Zn in males were lower than in females. Comparison of mean micronutrients showed that Cu and Zn were significantly (p<0.01) less in males than in females but Fe was not different. According to Fig. 1 TSH levels in male lambs were 36.3% higher than in females, Cu and Zn in females were 12.6% and 7.3% higher than in males, respectively (Fig. 2). The ratio of T3:T4 in males was 0.81, for females was 0.66 and for overall was 0.71 (Table 2). The above ratio was higher in males than females which was not significant (Fig. 3). Pearson correlation analysis between thyroid hormones and micronutrients showed that there was a significant negative relationship between T3/TSH r = -0.36) and T4/Cu (r = -0.24) in males but not in females. Overall, there was a significant negative relationship between TSH/T3 (r = -0.16). There was a positive and significant relationship between Cu/Zn (r = 0.38), Fe/Zn (r = 0.44) in males, females and all samples (p<0.01) but Cu and Fe were not related (Table 3).

DISCUSSION

The mean concentration of thyroid hormones in lambs of this study was about normal. The amount of T3 and T4 reported by Kozat[23] were 2.76 and 0.79, in pregnant ewes were 0.85 and 64.9 and in pregnant and lactating goats were 1.38 and 78.4 nmol/l, respectively[24] indicates that they are varies between breeds and age of animals. Thyroid hormones, especially T3, play an important role in the body's metabolism. T3 and T4 are affected by TSH which is secreted by the pituitary gland[25]. The functions of these hormones are almost the same, T3 in blood and fluids and T4 in the thyroid gland is high but in terms of speed and intensity of action, T3 is stronger than T4 and stays in the blood for a shorter time. Iodine and selenium (Se) are essential for the synthesis and metabolism of thyroid hormones in converting T4 to T3[11]. Blood Cu and Se levels directly affect thyroid activity[21, 3]. Ruminant feeding in pastures is generally associated with a deficiency of Cu, iodine and Se which affect the activity of thyroid hormones[8]. Therefore, aministration of trace minerals in forms of oral, injection, slow release, mineral fertilizers and mineral water in the breeding of small ruminants is inevitable[26]. The levels of Cu, Zn, iodine and Se must be balanced to regulate thyroid activity[27, 11]. Researchers acknowledged that iodine and Se are essential for the survival and growth of lambs[19]. In spite of the macro and trace minerals could affect thyroid hormones, the activity of T3 and T4 also contribute to a variety of factors including pregnancy[28], parturition, lactation[29] and poisoning[30] while Nazifi *et al*.[31] did not show daily changes in thyroid hormones following consecutive days of sampling.

In this study, the mean T3 of males was higher than in females and the T4 of females was higher than in males which was not significant and could indicate a long conversion of T4 to T3 in females or high consumption of T3 in females. Overall, about 93% of thyroid hormones is T4 and the rest is T3. However, all T4 is eventually converted to T3 by deiodinases in tissues, so, both T3 and T4 are functionally important[32]. T4 is a growth factor in lambs and basically, the growth of males is higher than that of females[33] which in this study may be the reason for high T3 consumption. Keles *et al*.[34] consider thyroid hormone deficiency as the most important factor in delaying calf growth. Since, Cu and Se deficiency have been reported in lambs[23], this can directly affect thyroid activity and reduce T3 in the long run[21]. Therefore, the low level of T4 in males is probably due to its rapid conversion to T3 for consumption for male growth. However, the difference between these hormones was not significant[17]. According to the results of this study, TSH was significantly higher in males than in females. TSH regulates the activity of T3 and T4 which is released by TRH[17]. TSH is directly inhibited by T4 and stimulated and increased by T3, so, a 36.3% increase in TSH may be in response to a slight decrease in T3 in males or conversely, a decrease in TSH is a reflection of a high T4 in lambs[13]. TSH has been reported to increase by several times in trace minerals deficiencies such as Se, following by decline T3 and enhance T4[35]. Therefore, supplementation of Se and nanoselenium will increase the concentration of thyroid hormones[36], although, some have suggested the opposite[37]. However, in Se deficiency, the T3:T4 ratio decreases, meaning that the males in this study are more likely to have Se deficiency[10].

In this study, the T3:T4 ratio in males (0.78) was not significantly higher than in females (0.67) and in all lambs (0.70) which were higher than the findings of Kozat[23] for lambs (0.35), Novoselec *et al*.[38] in pregnant and lactating ewes (0.0125). Determination of T3:T4 ratio in humans is a functional indicator of Se status[3]. The conversion of T4 to T3 can be assessed by monitoring the ratio of T3:T4 in the blood. Progressive reduction of this ratio in humans with Se supplementation can be reversed[3]. The relatively low ratio of T3:T4 in females means that the conversion of T4 to T3 is low and therefore, the amount of T4 in the blood of females will be higher than in males (Table 1). Konecny *et al*.[39] and Novoselec *et al*.[38] improved the above ratio in pregnant and lambing ewes by administering Se.

The mean serum Cu concentration of lambs was in the normal range of 10.3-2.3 μg/L[40] but was lower in males than in females. The reason for the difference is related to the growth difference between males and females, in which males grow higher, faster and need more Cu[33]. The role of Cu and its metalloenzymes including cytochrome oxidase, etc. are the most important Cu antioxidants that are vital and essential in lamb's life[41]. Therefore, its primary reduction will be associated with enzootic ataxia and pregnancy toxemia[2]. Serum Cu cooperates closely with iron[1] and is considered important in males in terms of meat quality and in females in terms of growth, milk production and reproduction[5]. Copper toghether with T4 is the most important factor in growth retardation in lambs[34, 3]. Copper with selenium is directly involved in thyroid activity[21]. Blasig *et al*.[42] showed that serum Cu was affected by thyroid hormones in lambs so that hypothyroid new borns were at risk for Cu deficiency. Nazifi *et al*.[43] showed that serum Cu and thyroid hormones were not different in consecutive sampling, have shown a stable status and did not have a competitive effect between them. Abdollahi *et al*.[28] reported that thyroid hormones and Cu levels decrease simultaneously during pregnancy, so that, T4 and Cu were low in twin pregnancy and T3 and Cu were low in pregnancy with one lamb. These findings indicate that the combined effect of Cu and thyroid hormones and the need to study them in lambs and ewes.

The mean serum Fe concentration in this study was higher than the normal range of 3.46-3.75 μg/L[40] which was in the optimal range. Iron levels were not different between males and females and were consistent with the findings of Silva *et al*.[40]. Iron is one of the effective trace minerals in hematopoiesis and has a positive relationship with serum Cu and a negative relationship with Zn[9] while in this study they were positive. Iron and Cu are vital for the synthesis of thyroid hormones[11] and their deficiencies are related with anemia and decreased physiological activity of lambs[8]. In this study, Fe showed no effects on tyroid hormones activity. Ferritin and homosiderin are the Fe storage compounds[44]. In addition, to severe anemia, low levels of these minerals are associated with other complications[2]. Iron is frequently affected by pregnancy, parturition, lactation and its significant reduction has been reported in bleeding and postpartum infections[28]. The role of serum Fe in thyroid hormones is unclear and there is no report of their interaction or coordination[31, 28].

The average Zn serum concentration of lambs was in the normal range of 12.3-18.5 μg/l[40] and was optimal for lambs. Males showed none significantly lower Zn than in females which is consistent with the results of Silva *et al*.[40]. Zinc is one of the micronutrients active in protein synthesis, carbohydrate metabolism and many biochemical reactions, its deficiency is associated with skin disorders, growth, production and reproduction[2]. Zinc deficiency has been reported following pregnancy toxemia, lambing and subclinical ketosis due to decreased appetite, fetal needs, colostrum and milk production[45]. Decreased co-occurrence of Zn and Cu is related with effects on production, reproduction and increased oxidative stress[46, 3]. The effect of Zn deficiency on BHB metabolism is due to impaired insulin secretion[47]. Zinc and thyroid hormones cause neonatal growth retardation[24]. The levels of Zn and thyroid hormones did not differ between the sampling times, their values were not constant and affected by each other[43] the same as found in this study except for TSH which had a negative effect. Thyroid hormone and Zn levels are affected by pregnancy[28], parturition and lactation[29]. Zinc and Cu are closely related to the secretion of T4 and TSH[3]. Zinc plays an important role in the conversion of T4 to T3[11]. Sampaio *et al*.[17] reported that in Zn-deficient ewes, T3 and T4 were low, meaning that Zn is directly related to thyroid hormones while not in this study. In contrast to the above findings, Kececi *et al*.[48] reported that Zn supplementation reduces thyroid hormones. Zinc may contrast with Cu, reducing one may cause an increase over another[9] while it was positive in this study. Thus, this information emphasizes on the use of Zn supplements in food to coordinates with thyroid hormones[12].

It was predictable to observe a negative relationship between T3 and TSH in males because TSH will increase when T3 decreases in the body[17] while the lack of such a relationship in females can not be justified but however, for the combination of lambs such a negative relationship is still partially present with the reduced coefficient from 0.36-0.16 which indicates the effectiveness of females in thid correlation. No relationship was observed between T3 and T4 or T4 with TSH indicating a direct function of these hormones. The existence of a negative relationship in T4/Cu contradicts the positively reported results of Hefnawy *et al*.[10]. Also, the negative relationship between TSH/Zn is not consistent with the positive results of Jain[1], Pop *et al*.[3] and Sampaio *et al*.[17]. One of the reasons for this difference is that their study was in adult's animals and human while the males of this study were <2 months old. Similar results of this study were reported by Blasig *et al*.[42] in ewes. Tajik *et al*.[49] also reported a positive relationship between tyroid hormones in buffalo and Nazifi *et al*.[30] showed no association at all. In this study, no effect or relationship was observed between thyroid hormones and Fe which can be two separate indices. The positive relationship between Cu/Zn and Fe/Zn in this study is in contradiction with the findings of Abdel-Mageed *et al*.[9] who reported a negative relationship. In any way, the positive relationship between trace minerals in this study indicates the coordination and support of each other in the physiological activity of the body.

CONCLUSION

The activity of thyroid hormones and trace minerals in lambs was in the desired range and no deficiencies were expected. T3 levels were higher in males and T4 levels in females but were not significant. Males TSH were significantly higher than in females. The amount of Cu and Zn in males was significantly lower than in females but Fe did not differ. The T3/T4 ratio was not significantly higher in males than in females. There was a significant negative relationship between T3/TSH, T4/Cu and T4/Zn in males but not in females. Overall, there was a significant negative relationship between TSH/T3. Significant positive relationships were found between Cu/Zn and Fe/Zn in males, females and for all samples but Cu/Fe was not correlated. Finally, male lambs need more concentration ot trace minerals and eventually more thyroid hormones than in females.

The thyroid hormones and micronutrient concentrations in lamb's blood were about the standard level. The amount of thyroid hormones and trace minerals with the exception of T3 were higher in females than in males. The T3:T4 ratio was higher in males than in females. Thyroid hormones were not correlated with trace minerals in males and females except between TSH/Zn and T4/Cu which were negative. There was a positive and significant relationship between Cu/Zn and Fe/Zn in males, females and for whole samples but Cu/Fe was not correlated. Finally, male lambs need more trace minerals amounts and eventually more thyroid hormones than females.

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