

## ORIGINAL ARTICLE

# Relationship between Thyroid Hormones and Blood Contents of Zinc and Copper of Sheep at Different Physiological Status

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### Abstract

This study was conducted to determine the correlation between the levels of thyroid hormones (T3 and T4) and the levels of blood contents of zinc (Zn) and copper (Cu) of 100 sheep from two different farms (50 sheep/each). Each group of them subdivided into 5 subgroups (ten animals/each). Blood samples were collected for estimating the concentrations of T3, T4, Cu and Zn. Results showed that T3 and T4 levels were significantly higher ( $p < 0.01$ ) in lambs than in other sheep in both groups (A and B). Serum levels were significantly lower ( $p < 0.01$ ) in males than in other females in group A and significantly higher ( $p < 0.01$ ) in lambs than in other adult in group B. Serum copper levels were significantly higher ( $p < 0.01$ ) in males than in group A. A strong linear positive correlation was found between blood Zn levels and T3 and T4 levels in the lambs of group A (significant at 0.01). A strong linear negative correlation was found between blood Zn levels and T3 and T4 levels in the males and non-pregnant of group A (significant at 0.01). The Cu levels in blood showed a strong linear positive correlation with T3 and T4 levels in the pregnant ewes of group A (significant at 0.01) and the males of group B (significant at 0.01). A strong linear negative correlation was observed between blood Cu levels and T3 and T4 levels in the non-pregnant ewes of both groups A and B (significant at 0.01).

### Keywords

Copper, Sheep, Thyroid hormones, Zinc

## 1. Introduction

Among domestic animals, the function and diseases of the thyroid gland are less known in livestock. Appropriate thyroid gland function and hormone activity are crucial to sustain the productive performance of domestic animals (i.e., milk, hair, and growth) (Chatterjea and Shinde, 2005).

Changes in the concentrations of thyroid hormones in blood are indirect indications of alterations in the activity of thyroid gland (Todini, 2007). The concentrations of these hormones are influenced by several factors, including age, season, climate, nutrition, breed, gender, ovarian endocrine function, other physiological factors (i.e., reproduction, pregnancy and lactation), and disease (Todini, 2007; Nazifi et al., 2008). The thyroid hormones are the most important humoral factors in the process of setting and modulating the basal metabolic rate in target tissues, as the liver, kidney, heart and brain (Saicic et al., 2006).

Previous research has determined the concentrations of thyroid hormones in the blood of sheep (Bekeova et al.,

1993), particularly in different growing and reproductive stages.

A normal thyroid status is relying on the presence of several trace elements required for the synthesis and metabolism of thyroid hormones. There are also some other trace elements such as copper, iron and zinc, although their function in the thyroid gland is less well distinct; however, a suboptimal or superoptimal dietary intake of all these elements can adversely affect the metabolism of thyroid hormones (Aurthor and Beckett, 1999).

Kececi and Keskin (2002) reported a significant negative correlation between the serum thyroid hormones and zinc concentration of erythrocytes in healthy male Merino lambs and Angora goats.

This study aimed to investigate the levels of T3 and T4 and some trace elements (Zn and Cu) at different physiological stages of sheep and to determine the correlation between the thyroid hormone levels and Zn and Cu levels in blood.

## 2. Materials and Methods

### 2.1. Animals

A total of 100 sheep from two different farms (50 sheep in each farm), including the farm of the Faculty of Veterinary Medicine, Beni-Suef University (group A), and a private farm in Beni-Suef province (group B), were used in this study. All animals were subjected to thorough clinical examination according to Radostits et al., (2000) and parasitological examination according to Urquhart (1996) and they were approved healthy and free from internal and external parasites. Each group of animals were subdivided into five subgroups (each containing ten animals) as follows:

**Group 1:** lambs (1-7 days)

**Group 2:** adult male (2-4 years)

**Group 3:** adult non-pregnant female animals (2-4 years)

**Group 4:** pregnant female animals (2-4 years)

**Group 5:** dams (lactating sheep) (2-4 years)

### 2.2. Diets

In summer, sheep in group B were fed daily with 1kg of a commercial concentrate mixture and approximately 0.5kg of *Berseem* hay. Rice straw was provided ad libitum. In winter, the animals were fed with the same diets, but with the substitution of hay with fresh *Berseem* (approximately 700g/ewe/day). Moreover, before the breeding season, the amount of the commercial concentrate mixture was increased up to 1.50kg/animal/day, whereas the amount of other ingredients remained the same. The commercial concentrate mixture contained 14% crude protein (CP), 15% crude fiber (CF), 9% ash, 12% moisture, 2% ether extract (EE), and 65% total digestible nutrients (TDN). Sheep in group A were fed on the commercial concentrate mixture and yellow corn. For this group, the commercial concentrate mixture contained 16% CP, 15% CF, 12% ash, 12% moisture, 2% EE, and 65% TDN. The diets were provided to the animals as a percentage of body weight according to the National Research Council (2007).

### 2.3. Samples

Blood samples were collected from each animal via jugular vein puncture in a clean dry centrifuge tube for harvesting non-hemolyzed serum. The samples were centrifuged at 3000rpm for 10min. The clear serum was separated carefully and stored in clean dry Eppendorf tubes at  $-20^{\circ}\text{C}$  for estimating the concentrations of thyroid hormones (T3 and T4) by duplicate determinations using commercial kits for clinical use in humans (Abbott Laboratories, USA) in an Imx-Abbott immune-analyzer and also for estimating the levels of Cu and Zn.

The serum levels of copper and zinc were estimated using the respective test kits supplied by Bio-diagnostic, according to methods described by Sharma and Singh (2009) and Abe et al., (1989) respectively.

### 2.4 Statistical Analysis

Data analyses were conducted using a statistical software program (SPSS for windows Version 16, SPSS Inc., Chicago, USA). ANOVA test was used to compare the

means between groups. Differences between mean values were considered to be significant at  $p < 0.01$ . The correlation between Zn and Cu and T3 and T4 levels was determined in the blood of all groups of animals.

## 3. Results and Discussion

In groups A and B, the levels of T3 and T4 (Table, 1) and Figs (1, 2) were significantly higher ( $p < 0.01$ ) in lambs than in other animals, except in non-pregnant females in group B. These results were consistent with those reported by Karapehliyan et al., (2007) and Josip et al., (2009) who showed that lambs had significantly ( $p < 0.01$ ) higher T3 and T4 concentrations than sheep of other age groups. The changes in these levels indicated that the lambs had a slightly increased metabolic activity.

The T3 and T4 levels were significantly higher ( $p < 0.01$ ) in males and pregnant females than in dams and non-pregnant females in group A. However, in group B, the levels of T3 and T4 were significantly higher ( $p < 0.01$ ) in adult non-pregnant females than in other animals but significantly lower ( $p < 0.01$ ) in pregnant females than in other animals.

When we compared groups A and B, we found significantly higher ( $p < 0.01$ ) T3 and T4 levels in lambs, males, and pregnant females in group A than those in group B. However, the levels were significantly lower ( $p < 0.01$ ) in adult non-pregnant females in group A than those in group B. In dams, there was a minor fluctuation in the levels of T3 and T4 between the two groups, which may be due to differences in age, sex, breed, and ration. Josip et al., (2009) also reported significantly lower ( $p < 0.01$ ) T3 and T4 concentrations in the blood of lactating sheep than in the blood of non-pregnant and pregnant sheep. These results were consistent with those reported by Colavita et al., (1983) who mentioned that growing goat kids had higher thyroid hormone levels in their blood than adults. However, the results of the present study were not consistent with those reported by El-Barody et al., (2002) who indicated a decrease of T4 concentration in lambs with increasing age and Todini et al., (2007) who reported increased T4 activity in the blood of lactating goats in relation to late pregnancy.

Regarding serum zinc levels, in Table (2) and Fig. (3), the values were significantly lower ( $p < 0.01$ ) in males ( $96.2 \pm 9.9$ ) than in other animals in group A. However, the Zn levels were significantly higher ( $p < 0.01$ ) in lambs ( $132.5 \pm 1.4$ ) than in other animals in group B. These data were consistent with the results recorded by Jeffery (2006) who mentioned that the "normal range" for Zn and Cu levels in body storage tissues would be higher in early neonates than in an adult animal, which may be attributed to the accumulation of these minerals in fetuses at different rates during gestation, necessitating adequate aging of the fetus for interpretation. Moreover, these minerals, for which little is provided in milk, accumulate at higher concentrations during gestation to provide neonates with adequate body reserves for survival until they begin foraging. When we compared the two groups, we found that the levels were significantly higher ( $p < 0.01$ ) in lambs and males in group B than in group A, but

significantly lower ( $p < 0.01$ ) in pregnant females and dams in group B than in group A, which may be attributed to differences in the feeding regimen.

Regarding serum Cu levels in **Table (2)** and **Fig. (4)**, the values were significantly higher ( $p < 0.01$ ) in males ( $20.3 \pm 1$ ) than in other animals in group A. However, the levels were significantly higher ( $p < 0.01$ ) in non-pregnant females ( $18.1 \pm 2.7$ ) than in other animals in group B. These results were consistent with those recorded by **Chhabra (2006)** who reported that there was no effect of age on blood Cu levels. When we compared the two groups, we found that serum Cu levels were significantly lower ( $p < 0.01$ ) in the animals of group B than in the animals of group A, which may be due to differences in ration and breed between the two farms.

Regarding the relationship between Zn levels in blood and T3 and T4 levels in all groups of animals (**Table, 3**), we found no significant linear correlation between these parameters in the dams of group A and the males and non-pregnant animals of group B. However, there was a strong linear positive correlation between Zn levels in blood and T3 and T4 levels in the lambs of group A (significant at 0.01) and the lambs and dams of group B (significant at 0.05). These data were in agreement with those recorded by **Kucharzewski et al., (2003)** and **El-sisy et al., (2008)** who reported that zinc deficiency in the body may lead to decreased secretion of thyroid hormones, which affects the normal metabolism of the body and the resting metabolic rate. They further mentioned that zinc deficiency is associated with enhanced expression of hepatic thyroxine-5'-monodeiodinase that catalyzes the inactivation of thyroid hormones. Our results are also consistent with those reported by **Aihara et al., (1984)** who mentioned that patients with hyperthyroidism show increased urinary excretion of zinc and that consumption of high amounts of zinc can contribute to hyperthyroidism because zinc acts as a stimulator of the thyroid gland.

The well-known effect of zinc on some endocrine glands for example the master gland, the pituitary and on the hypothalamus is that it plays a role in the synthesis of

hormones as thyrotropin-releasing hormone (**Brandao Neto et al., 2006**).

We found a strong linear negative correlation between Zn levels in blood and T3 and T4 levels in the males and non-pregnant animals of group A (significant at 0.01) and the pregnant ewes of both groups A and B (significant at 0.05). These results were similar to those reported by **Pfeiffer and Braverman (1982)** who showed a significant negative correlation between the serum thyroid hormones and zinc concentration of erythrocytes. Similar results were also achieved by **Kececi and Keskin (2002)** in healthy male Merino lambs and Angora goats.

Regarding the relationship between Cu levels in blood and T3 and T4 levels in all groups of animals (**Table, 4**), we found no significant linear correlation between these parameters in the lambs and dams of group A. There was also no significant linear correlation between Cu levels in blood and T4 levels in the males of group A and the pregnant ewes and dams of group B. However, a strong linear positive correlation was found between Cu levels in blood and T3 and T4 levels in the pregnant ewes of group A (significant at 0.01) and males of group B (significant at 0.01). There was also a strong linear positive correlation between Cu levels in blood and T3 levels in the dams of group B (significant at 0.05) and the lambs of group B (significant at 0.01), but there was a moderate linear positive correlation between Cu levels in blood and T4 levels in the lambs of group B.

A strong linear negative correlation was found between Cu levels in blood and T3 and T4 levels in the non-pregnant ewes of both groups A and B (significant at 0.01) and the males of group A (with T3 levels alone) (significant at 0.05). These results were consistent with those reported by **Baltaci et al., (2013)** who found that relatively higher levels of Cu were associated with hypothyroidism and relatively lower levels of Cu were associated with hyperthyroidism. Similarly, **Meeker et al., (2009)** also observed a monotonic decrease in the levels of thyroid-stimulating hormone with an increase in the levels of serum Cu.

**Table 1.** Levels of thyroid hormones (T3 and T4) in sheep, groups A and Group B (mean  $\pm$  S.D).

		Lambs	Male	Non-pregnant female	Pregnant female	Dam (ewes)
T3 ng/dl	Group A	180 $\pm$ 19.6 <sup>a</sup>	149.6 $\pm$ 21.4 <sup>b</sup>	114.2 $\pm$ 15.9 <sup>c</sup>	147.8 $\pm$ 26.5 <sup>b</sup>	124.5 $\pm$ 8.5 <sup>c</sup>
	Group B	143.4 $\pm$ 5.8 <sup>a,b</sup>	131.6 $\pm$ 4.7 <sup>b,c</sup>	146.9 $\pm$ 22.2 <sup>a</sup>	116.8 $\pm$ 15.7 <sup>d</sup>	130.2 $\pm$ 9.6 <sup>c</sup>
T4 ug/dl	Group A	12.4 $\pm$ 1.7 <sup>a</sup>	10.6 $\pm$ 1.7 <sup>b</sup>	6.9 $\pm$ 0.6 <sup>c</sup>	9.7 $\pm$ 2.3 <sup>b</sup>	7.4 $\pm$ 1.0 <sup>c</sup>
	Group B	9.7 $\pm$ 1.1 <sup>a</sup>	8.0 $\pm$ 0.6 <sup>b</sup>	9.7 $\pm$ 1.8 <sup>a</sup>	7.4 $\pm$ 0.8 <sup>b</sup>	8.1 $\pm$ 0.8 <sup>b</sup>

Mean values with different letters in the same row are significant at  $p < 0.01$ .

**Table 2.** The levels of zinc (Zn) and copper (Cu) in sheep in sheep, groups A and Group B (mean  $\pm$  S.D).

		Lambs	Male	Non-pregnant female	Pregnant female	Dam
Zn Ug/dl	Group A	98.8 $\pm$ 7.0 <sup>a,b</sup>	96.2 $\pm$ 9.9 <sup>b</sup>	108.0 $\pm$ 15.3 <sup>a</sup>	105.2 $\pm$ 7.4 <sup>a,b</sup>	104.6 $\pm$ 9.7 <sup>a,b</sup>
	Group B	132.5 $\pm$ 1.4 <sup>a</sup>	106.2 $\pm$ 11.8 <sup>b</sup>	105.4 $\pm$ 8.6 <sup>b</sup>	98.2 $\pm$ 0.9 <sup>c</sup>	88.9 $\pm$ 1.7 <sup>d</sup>
Cu Mmol/l	Group A	13.7 $\pm$ 1.3 <sup>c</sup>	20.3 $\pm$ 1.0 <sup>a</sup>	18.9 $\pm$ 2.8 <sup>a,b</sup>	18.8 $\pm$ 0.9 <sup>b</sup>	11.9 $\pm$ 0.7 <sup>d</sup>
	Group B	12.8 $\pm$ 0.9 <sup>c</sup>	14.7 $\pm$ 3.0 <sup>b</sup>	18.1 $\pm$ 2.7 <sup>a</sup>	11.7 $\pm$ 1.2 <sup>c,d</sup>	10.6 $\pm$ 0.7 <sup>d</sup>

Mean values with different letters in the same row are significant at  $p < 0.01$ .

**Table 3.** Results of the correlation between zinc levels and T3 and T4 levels in the blood.

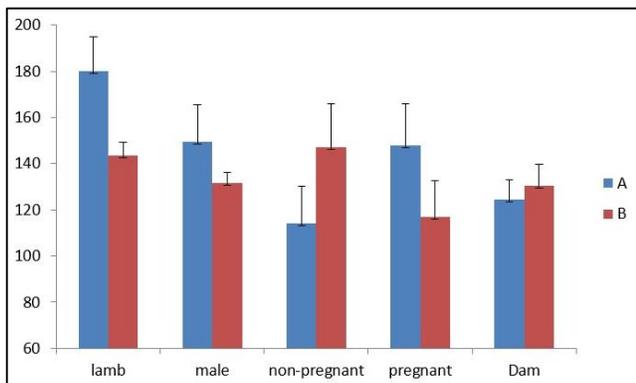
Groups of animals	T3 (r (correlation coefficient))	T4 (r (correlation coefficient))
Lamb (Group A)	0.987**	0.987**
Lamb (Group B)	0.663*	0.576
Male (Group A)	-0.826**	-0.794**
Male (Group B)	-0.196	-0.100
Non-pregnant (Group A)	-0.846**	-0.848**
Non-pregnant (Group B)	-0.513	-0.586
Pregnant ewe (Group A)	-0.743*	-0.743*
Pregnant ewe (Group B)	-0.721*	-0.670*
Dam (Group A)	0.191	0.135
Dam (Group B)	0.642*	0.584

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

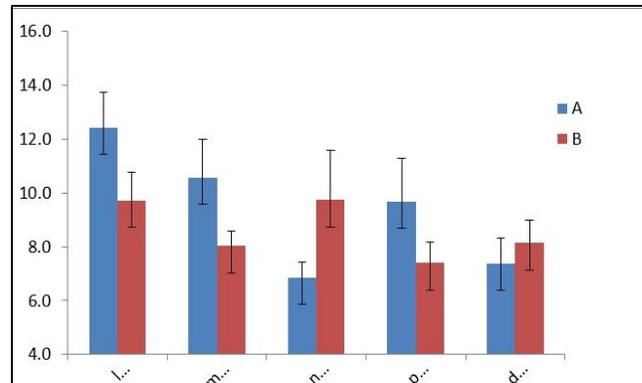
**Table 4.** Results of the correlation between copper levels and T3 and T4 levels in the blood.

Groups of animals	T3 (r (correlation coefficient))	T4 (r (correlation coefficient))
Lamb (Group A)	0.123	0.059
Lamb (Group B)	0.740*	0.598
Male (Group A)	-0.724*	0.018
Male (Group B)	0.788**	0.815**
Non-pregnant (Group A)	-0.873**	-0.886**
Non-pregnant (Group B)	-0.836**	-0.870**
Pregnant ewe (Group A)	0.828**	0.824**
Pregnant ewe (Group B)	-0.463	-0.426
Dam (Group A)	-0.228	-0.076
Dam (Group B)	0.661*	0.501

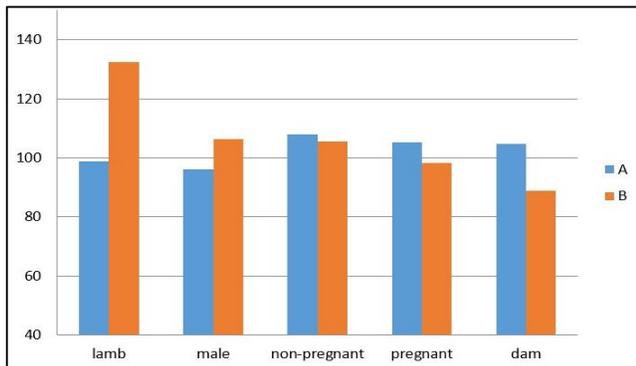
\* Correlation is significant at the 0.05 level (2-tailed). \*\* Correlation is significant at the 0.01 level (2-tailed).



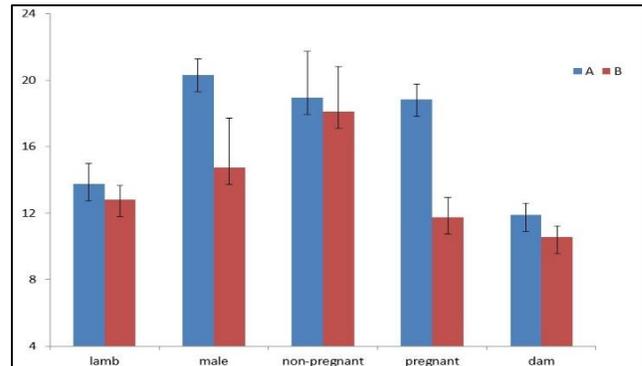
**Fig. 1.** Serum T3 levels in sheep, groups A and B



**Fig. 2.** Serum T4 levels in sheep, groups A and B



**Fig. 3.** Serum zinc levels in sheep, groups A and B



**Fig. 4.** Serum copper levels in sheep, groups A and B

### 4. Conclusion

A correlation was found between the levels of thyroid hormones (T3 and T4) as well as (zinc and copper) in the blood of sheep at different physiological status.

### 5. Author Contributions

All authors contributed equally to the study including design of the experiments, methodologies, analysis and interpretation of results and drafting the manuscript.

## 6. Conflict of Interest

The authors declare no conflict of interest.

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