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ZINC SUPPLEMENTATION DECREASES TOTAL THYROID HORMONE CONCENTRATION IN SMALL RUMINANTS

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The effect of dietary zinc (Zn) supplementation on plasma Zn and serum thyroid hormones was evaluated in healthy male Merino lambs and Angora goats. A total of 12 lambs and 12 goats were divided into two equal groups as control and Zn groups in separate experiments. The lambs and goats of the control groups were fed basal rations alone. The Zn contents of these rations prepared for lambs and goats were 40 mg/kg and 35 mg/kg in dry matter (DM), respectively. Both species of animals in the Zn groups were fed a basal ration supplemented with zinc sulphate adjusted to 250 mg Zn/kg diet in DM. The feeding trial lasted for 12 weeks in lambs and 8 weeks in goats. Blood samples were taken from the jugular vein at 4-week intervals. Both animal species in the Zn groups had higher plasma Zn values than the controls throughout the experimental period, except in the 4th week in goats. However, the levels of serum total thyroxine (T_4) and triiodothyronine (T_3) were lower in the lambs and goats of the Zn groups, except in the 4th week, as compared to those in the controls. Moreover, serum total thyroid hormone levels of the goats were higher at the 4th week than at the 8th week. Although there was a decrease in the levels of free thyroxine and triiodothyronine of both small ruminant species in the Zn groups when compared to the controls, these alterations were not statistically significant. These results may show that zinc supplementation to the diet at this dose reduces total thyroid hormone levels in small ruminants but does not yet impair the euthyroid status of the organism.

Key words: Zinc, thyroxine, triiodothyronine, lamb, goat

The significance of the thyroid gland in the organism is well known, and several factors such as certain trace metals may influence the metabolism of thyroid hormones. Until recently a relationship between trace elements and thyroid hormone metabolism has only been described for iodine, which is crucial for the formation of hormones in the thyroid gland (Neve, 1992). Zinc also may play important roles in thyroid metabolism. Thus, its low (Gupta et al., 1997; Ruz et al., 1999) or excessive (Dean et al., 1991) levels may cause numerous abnormalities. Therefore, it has been emphasised that excessive dietary zinc intake re-

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sulted in disturbances in serum thyroid hormone levels in man (Miyamoto et al., 1991) and animals (Dean et al., 1991).

An interaction between thyroid hormones and zinc metabolism has been reported, and high serum zinc values have been noted in patients with hyperthyroidism (Nishi et al., 1980; Aihara et al., 1984). Furthermore, serum zinc content was found to be reduced in hypothyroid pigs (Schone et al., 1990). Excess zinc has been found to decrease the levels of serum thyroxine (T_4) and triiodothyronine (T_3) in poultry (Dean et al., 1991) and in humans (Nishiyama et al., 1994).

Since subclinical zinc deficiency is thought to be very widespread, zinc is often added to the diets of livestock (McDowell, 1992). In addition, naturally occurring zinc deficiency has been reported to influence thyroid status in humans (Morley et al., 1981). Considering these facts, we thought that the effect of zinc on the metabolism of the thyroid gland should be understood better in order to expand the existing knowledge concerning thyroid physiology. Thus, the aim of the present study was to investigate the effect of feeding nutritional levels of zinc on plasma zinc and serum thyroid hormone concentrations in healthy Merino lambs and Angora goats.

Materials and methods

The experiment was conducted using clinically healthy 12 Merino ram lambs (14 weeks old and weighing 26.0 ± 2.1 kg at the start of the study) and 12 male Angora goats (12 months old and weighing 18.2 ± 2.4 kg at the start of the study) obtained from the Animal Research Institute and commercial breeders in Konya, respectively. The guiding principles in the care and use of animals were observed. The lambs and goats were housed indoors in individual pens under natural lighting, and animals of both species were randomly and separately allocated to two equal groups, i.e. control and Zn-supplemented groups.

The commercial basal diet was formulated to meet the National Research Council (1985) requirements (for lambs; 93.27% dry matter (DM), and 18.93% crude protein, 11.82% crude fibre, 1.77% crude fat, 5.57% ash and 9.16 MJ ME/kg in DM basis, for goats; 90.34% DM, and 12.53% crude protein, 8.25% crude fibre, 3.21% crude fat, 5.80% ash and 9.00 MJ ME/kg in DM basis). The control animals were fed the basal diets alone and the basal diet of the lambs and goats contained 40 mg Zn/kg and 35 mg Zn/kg, respectively. On the other hand, both ruminant species of the Zn group were fed the basal diet supplemented with zinc sulphate ($ZnSO_4 \times 7\ H_2O$, Merck) adjusted to 250 mg Zn/kg diet in DM basis. Zn amounts in DM of the rations of all groups were controlled and calculated before the trial. The experimental diets and drinking water were provided *ad libitum* throughout the experimental period. The feeding trial lasted for 12 weeks in lambs and 8 weeks in goats.

Blood samples were obtained from the lambs and goats by venipuncture of the jugular vein between 8:00 and 8:30 a.m. and collected in low metal vacuum tubes (vacutainers, BD6527, Becton Dickinson, Rutherford) at 4-week intervals. Blood was transferred to a test tube with no anticoagulant to obtain serum and to a container containing sodium heparin as anticoagulant to obtain plasma. Both serum and plasma were immediately frozen at –20 °C until analysis.

The Zn levels of plasma and diets were measured using an atomic absorption spectrophotometer (Bulc Scientific 200A; Perkin Elmer Corporation, 1982). Serum T_3 and T_4 (total and free) concentrations were determined using the radioimmunoassay method previously described by Anderson et al. (1988).

Data for parameters were grouped and expressed as mean ± pooled standard errors of means. Differences between the means of control and Zn groups in both ruminant species, and between the means at 4th and 8th weeks in the goats were identified by Student's *t*-tests. The means obtained at the 4th, 8th and 12th weeks in the lambs were subjected to analysis of variance, if appropriate ($P < 0.05$), post hoc analyses were carried out using the Duncan's test for multiple comparisons. Statements of statistical significance are based on $P < 0.05$ (SPSS, 1988).

Results

As shown in Tables 1 and 2, at the end of the 4th week of the experiment, there were no significant alterations in the investigated parameters of either small ruminant species ($P > 0.05$), with the exception of the increased plasma Zn value found for lambs in the Zn group. However, at the other sampling times of the study, animals in the Zn groups had significantly higher mean plasma Zn values than the control ones ($P < 0.05$). Also, serum total thyroxine (TT_4) and triiodothyronine (TT_3) concentrations were significantly lower in the lambs and goats of the Zn groups as compared to those in the controls. Furthermore, serum TT_4 and TT_3 concentrations of the goats in the Zn group were higher at the 4th week than at the 8th week ($P < 0.05$). Zinc treatment did not significantly affect serum free thyroxine (FT_4) and triiodothyronine (FT_3) levels ($P > 0.05$).

Although zinc-supplemented animals showed generally significant differences in their plasma zinc, serum TT_4 and TT_3 concentrations when compared to the control animals, except for the 4th week, these altered values were in the normal reference range.

Discussion

The various metabolic and growth-depressing effects of excess Zn in many animal species have been reviewed (McDowell, 1992), and several studies indicate that moderately excessive Zn intakes, typically used with self-

supplementation, can have adverse metabolic consequences in humans (Nishiyama et al., 1994) and animals (Dean et al., 1991). It has been reported that the minimum zinc level of the diet must be 20–33 mg Zn/kg diet in ruminants (National Research Council, 1985; McDowell, 1992), and the toxic level of zinc was found to be 1000 mg zinc/kg diet for sheep and goats (Ensminger and Parker, 1986). Moreover, it was declared that zinc tolerance level was 300 mg zinc/kg diet for sheep (Reid et al., 1987). Zinc levels used in the diets of lambs and goats of this study were close to those regarded as tolerable in sheep and goats (Ensminger and Parker, 1986; Reid et al., 1987).

Table 1

Effect of dietary zinc (Zn) treatment on plasma Zn and serum total thyroxine (TT_4), total triiodothyronine (TT_3), free thyroxine (FT_4), and free triiodothyronine (FT_3) levels in Merino lambs[1]

Investigated parameters[*]	Sampling time	Groups		Significance[4]
		Control[2]	Zn group[3]	
Zn (μg/ml)		1.16 ± 0.05	1.44 ± 0.07	+
TT_4 (μg/dl)		6.58 ± 0.24	6.10 ± 0.29	NS
TT_3 (ng/dl)	4th week	97.12 ± 2.49	90.61 ± 2.27	NS
FT_4 (ng/dl)		1.78 ± 0.07	1.54 ± 0.10	NS
FT_3 (pg/ml)		2.17 ± 0.12	2.05 ± 0.11	NS
Zn (μg/ml)		1.12 ± 0.04	1.47 ± 0.05	+
TT_4 (μg/dl)		6.67 ± 0.27	5.90 ± 0.15	+
TT_3 (ng/dl)	8th week	97.62 ± 2.27	82.54 ± 4.12	+
FT_4 (ng/dl)		1.75 ± 0.10	1.50 ± 0.12	NS
FT_3 (pg/ml)		2.18 ± 0.10	2.00 ± 0.12	NS
Zn (μg/ml)		1.16 ± 0.05	1.61 ± 0.07	+
TT_4 (μg/dl)		6.65 ± 0.30	5.84 ± 0.17	+
TT_3 (ng/dl)	12th week	97.80 ± 2.34	83.20 ± 4.37	+
FT_4 (ng/dl)		1.80 ± 0.08	1.45 ± 0.14	NS
FT_3 (pg/ml)		2.20 ± 0.14	1.90 ± 0.16	NS

[1]Values represent the means ± SEM of two groups of 6 merino lambs; [2]Control group fed basal ration alone (40 mg Zn/kg diet in DM basis); [3]Zn supplemented as $ZnSO_4$ (250 mg Zn/kg diet in DM basis); [4]Values within rows with NS: not significant ($P > 0.05$) and + :$P < 0.05$ between control and Zn groups, according to Student's *t*-tests; [*]Significant differences were not found among the values of the same parameters within columns ($P > 0.05$), according to Duncan's multiple range tests

It has been reported that normal plasma zinc values for sheep and goats are in the range of 0.80–1.20 μg/ml (Rushton, 1984), and that minimum plasma zinc value must be between 0.60 μg/ml and 0.80 μg/ml (McDowell et al., 1993). Plasma zinc concentration of the control lambs in the present study was in the range of normal plasma zinc values reported by Rushton (1984), although in lambs of the Zn group it was slightly higher than that. On the other hand, it was found that the plasma zinc value of control goats was similar to normal zinc lev-

els reported for Angora goats by Reuter et al. (1987) and Niekerk et al. (1990) as 0.62 μg/ml and 0.72 μg/ml, respectively. However, these values are slightly lower than those declared by Rushton (1984). In addition, the plasma zinc levels of goats fed the zinc-supplemented ration were close to those obtained by Rushton (1984) but were higher than the zinc levels reported as normal by Niekerk et al. (1990) and Reuter et al. (1987).

Table 2

Effect of dietary zinc (Zn) treatment on plasma Zn and serum total thyroxine (TT_4), total triiodothyronine (TT_3), free thyroxine (FT_4), and free triiodothyronine (FT_3) levels of Angora goats[1]

Investigated parameters*	Sampling time	Groups		Significance[4]
		Control[2]	Zn group[3]	
Zn (μg/ml)		0.73 ± 0.08	0.94 ± 0.07	NS
TT_4 (μg/dl)		15.12 ± 0.52	14.32 ± 0.43	NS
TT_3 (ng/dl)	4th week	252.32 ± 5.28	242.24 ± 6.32	NS
FT_4 (ng/dl)		2.96 ± 0.20	2.84 ± 0.18	NS
FT_3 (pg/ml)		5.61 ± 0.32	5.54 ± 0.29	NS
Zn (μg/ml)		0.72 ± 0.02	1.01 ± 0.04	+
TT_4 (μg/dl)		15.23 ± 0.48	11.33 ± 0.37*	+
TT_3 (ng/dl)	8th week	256.28 ± 5.44	188.67 ± 7.44*	+
FT_4 (ng/dl)		2.98 ± 0.19	2.67 ± 0.10	NS
FT_3 (pg/ml)		5.74 ± 0.24	4.89 ± 0.30	NS

[1]Values represent the means ± SEM of two groups of 6 Angora goats; [2]Control group fed basal ration alone (35 mg Zn/kg diet in DM basis); [3]Zn supplemented as $ZnSO_4$ (250 mg Zn/kg diet in DM basis); [4]Values within rows with NS: not significant ($P > 0.05$) and + :$P < 0.05$ between control and Zn groups, according to Student's *t*-tests; *Values within columns are significantly different from the same parameters at 1st month, according to Student's *t*-tests

Large oral doses of zinc greatly increase whole blood and plasma zinc concentrations in livestock (McDowell, 1992). Sheep (Grace and Lee, 1992) and Angora goats (Eryavuz, 1999) given incremental dietary zinc supplements displayed mean plasma zinc increases. Similarly, our findings reveal that plasma zinc concentrations were higher in the zinc-supplemented lambs and goats than in the control animals, with the exception of the unchanged values found in the goats at the 4th week. There was an increase in plasma zinc level of goats in the Zn group at the end of the 4th week, but this increase was not statistically significant. Our report is consistent with other studies showing that zinc supplementation of the ration increased plasma zinc levels of Angora goats (Reuter et al., 1987; Eryavuz, 1999), sheep (Grace and Lee, 1992), and calves (Kincaid et al., 1997).

Deficiency (Henkin, 1976) or excessive level (Dean et al., 1991; Nishiyama et al., 1994) of zinc has been observed to affect the endocrine system adversely. It has been reported that the serum or plasma levels of thyroid hormones were reduced by high Zn intake (Dean et al., 1991; Nishiyama et al., 1994) and

were unchanged (Fujimoto et al., 1986) or increased (Oliver et al., 1987) on low Zn intake. In addition, the level of plasma zinc has been found to be correlated with plasma T_3 and T_4 levels (Nishi et al., 1980; Aihara et al., 1984). However, Morley et al. (1981) found no correlation between serum zinc and T_4 level in alcoholic cirrhotic patients. Zinc, in addition to its participation in protein synthesis, is involved in T_3 binding to its nuclear receptor (Miyamoto et al., 1991). Moreover, Dean et al. (1991) have claimed that high Zn intake may alter the production and secretion of thyroid hormones. They suggested that decreased thyroid function was due to diminished adenohypophyseal regulation of the thyroid gland, and that the depressed levels of circulating thyroid hormones may indicate hypothyroidism induced by zinc toxicity. Therefore, excessive dietary zinc intake has been noted to result in disturbances in the blood thyroid hormones in man (Nishiyama et al., 1994) and animals (Dean et al., 1991). In our study, although there were no significant differences in the serum thyroid hormone concentrations of lambs and goats of either group at the end of the 4th week, there was a decrease in the levels of TT_4, TT_3, FT_4 and FT_3 in both small ruminant species in the Zn groups when compared to controls at the other sampling times except the 4th week. However, only the serum TT_4 and TT_3 values were significantly lower in the lambs and goats of the Zn groups than in the controls. Furthermore, there were no statistically significant differences between the values found at the 4th and 8th weeks in goats and among those found at the 4th, 8th and 12th weeks in lambs, with the exception of the decreased serum TT_4 and TT_3 levels of the goats in the Zn group at the 8th week. The decreased serum total thyroid hormone levels of both ruminant species in the Zn groups are still within the normal reference range for small ruminants (Anderson et al., 1988), probably because the applied zinc supplementation was not of toxic level. In addition, the serum FT_4 and FT_3 levels of both animal species in the Zn groups were numerically reduced compared to the control ones, although these alterations were not statistically significant. Thus, these findings may indicate that zinc supplementation of the diets of small ruminants exerts an effect on thyroid hormone level but does not cause hypothyroidism affecting the entire organism, because the free thyroid hormone level, which determines the hormone supply of cells, is still normal (Braverman and Utiger, 1991).

Under the present experimental conditions, this study showed that the plasma zinc and serum TT_4 and TT_3 were significantly influenced by dietary zinc supplementation. The alterations of plasma zinc (Reuter et al., 1987; Niekerk et al., 1990) and serum TT_4 and TT_3 (Anderson et al., 1988) levels were in the normal range, probably because the applied zinc supplementation (250 mg Zn/kg diet) was close to the level regarded as tolerable in goats and sheep (Ensminger and Parker, 1986; Reid et al., 1987). Therefore, zinc supplementation may be used if it is necessary for other reasons, as it reduces the total hormone level but does not yet impair the euthyroid status of the organism (Braverman and Utiger,

1991). Consequently, although the present study is not able to explain all the changes observed, it shows that acceptable zinc addition to the ration at this dose does not significantly alter the thyroid function in small ruminants.

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