

Effects of Sulfur Supplementation on Thyroid Hormones in Angora Goats Fed With A High-Nitrate Diet[#]

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ABSTRACT

This study was to aimed for determination the effect of high dietary nitrate intake and inorganic sulfur supplementation on thyroids hormones in Angora goats. In this study, eighteen male Angora goats aged 14 months were divided into three groups ($n = 6$): control group fed with a basal diet, nitrate group fed with a basal diet supplemented with 1500 ppm nitrate, and nitrate + sulfur group fed with a basal diet supplemented with 1500 ppm nitrate and 1.8% sodium sulfate. On days 45, 90, 135, and 180 of the study, the concentrations of the thyroid-stimulating hormone (TSH), total and free triiodothyronine (TT₃ and FT₃), and tetraiodothyronine (TT₄ and FT₄) were measured in the serum samples. On day 180, except for TT₄, the serum TSH and total and free T₃ and free T₄ concentrations were higher ($P < 0.05$) in the nitrate + sulfur group than in the control and nitrate groups. This study suggested that Angora goats could tolerate a feed containing 1500 ppm nitrate with respect to the thyroid hormones, and inorganic sulfur might serve as a natural source for alleviating the negative effects of the high-nitrate diet on the thyroid gland in a dose-dependent manner.

Keywords: Angora goat, Nitrate, Sulfur, Thyroid hormones

Yüksek Nitratlı Diyetle Beslenen Ankara Keçilerinde Tiroid Hormonları Üzerine Kükürt İlavesinin Etkileri[#]

ÖZ

Bu çalışma, yüksek diyetli nitrat alımı ve inorganik kükürt ilavesinin Ankara keçilerindeki tiroid hormonlarına etkisini belirlemek amacıyla yapıldı. Çalışmada, yaklaşık 14 aylık yaştaki 18 erkek Ankara keçisi 3 gruba bölündü: kontrol grup bazal diyetle beslendi, nitrat grubu 1500 ppm nitrat ilave edilen bazal diyetle beslendi ve nitrat+kükürt grubu 1500 ppm nitrat ve %1.8 sodyum sülfat ilave edilen bazal diyetle beslendi. Çalışmanın 45., 90., 135. ve 180. günlerinde serum örneklerinde tiroid uyarıcı hormon (TSH), total ve serbest triiyodotironin (TT₃ ve FT₃) ve tetraiyodotironin (TT₄ ve FT₄) düzeyleri ölçüldü. 180. günde, TT₄ hariç, serum TSH, total ve serbest T₃ ve serbest T₄ konsantrasyonları nitrat+kükürt grubunda kontrol ve nitrat grubundan daha yüksekti ($P < 0.01$). Bu çalışma, Ankara keçilerinin tiroid hormonları dikkate alındığında 1500 ppm nitrat içeren bir beslemeyi tolere edebileceğini ve inorganik kükürdün doza bağlı olarak tiroid hormonları üzerine yüksek nitratlı diyetin olumsuz etkilerini hafifletmek için doğal bir kaynak görevi görebileceğini önermektedir.

Anahtar Kelimeler: Ankara keçisi, Kükürt, Nitrat, Tiroid hormonları

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INTRODUCTION

The use of high-nitrate fertilizers to increase agricultural production can lead to accumulation of nitrates/nitrites in soil, plants, and drinking water sources, and thus cause a potential health risk for humans and animals (Jordao et al. 2002, Mensinga et al. 2003). Generally, forage containing up to 1000 ppm nitrate is considered safe for ruminants, but forages containing 1200-1500 ppm nitrate cause chronic nitrate poisoning in the ruminants (Kaya and Akar 1989). Previous studies demonstrated that ruminants consuming 800-2000 ppm nitrate daily through water and forage showed subacute and chronic toxicity symptoms (Bartik and Piskač 1981, Pirinçci and Acet 1984). Although nitrates are relatively nontoxic for the ruminants, nitrites are highly toxic. In ruminants, under normal conditions, nitrate is first reduced to nitrite (Chow and Hong 2002, Takahashi et al. 1998), and then to ammonia by the microorganisms in the rumen. However, because the conversion of nitrate into nitrite is faster than the reduction of nitrite to ammonia by the microorganisms, a high nitrate intake through water and diet leads to the accumulation of nitrite in the rumen (Cheng et al. 1988).

Goitrogenic agents such as nitrate, perchlorate, and thiocyanate act as competitive inhibitors of the Na⁺/I⁻ symporter (Jahreis et al. 1986). Therefore, nitrate reduces inorganic iodine uptake by the thyroid gland and, thus, intrathyroidal iodine concentration. When the animals consume water or a diet with high nitrate levels, blockage of the Na⁺/I⁻ transport protein decrease the synthesis of thyroid hormones (Kostogryz ve ark. 1989, Nlend ve ark. 1999). Previous studies indicated that high nitrate intake decreased the binding of active iodine to Na⁺/I⁻ transport protein and thyroid gland activity in humans and animals (Bruning-Fann and Kaneene 1993, Eskiocak et al. 2005, Simon et al. 2000).

Studies on small ruminants revealed that chronic nitrate toxicity can decrease thyroid hormone concentration in sheep (Georgiev et al. 1987) and goats (Simon et al. 2000). Although a few studies exist on the thyroid hormones in Angora goats (Keçeci and Keskin 2002, Puchala et al. 2001) to date no study has demonstrated the effects of high dietary nitrate intake on the thyroid hormones in Angora goats producing mohair.

Sulfur is a constituent of several biomolecules such as proteins, nucleic acids, and sulfur-containing coenzymes. It is particularly required in ruminants for microbial synthesis of sulfur-containing amino acids (cysteine, cystine, and methionine), thiamine,

biotin, and enzymes (Carneiro et al. 2000, Takahashi et al. 1989). Moreover, nitrate supplementation increased sulfur requirements in the diet to improve microbial synthesis of sulfur-containing amino acids in the rumen. The nitrogen/sulfur ratio in the diets of ruminants is 10/1 (National Research Council, 1981). Several studies reported that adding easily digestible carbohydrates or sulfur to diet decreased the nitrite accumulation due to an increased amount of ammonia used by bacteria (Burrows et al. 1987, Takahashi et al. 1998, Takahashi et al. 1989). Given that nitrate is rapidly converted into nitrite by microorganisms in the rumen, sulfur supplementation to a high-nitrate diet may promote conversion rate of nitrite into ammonia and also decrease the accumulation of nitrite in the rumen. Takahashi et al. (1989) suggested that sulfur supplementation to the diet of ruminants decreases the formation of nitrite by microorganisms in the rumen.

In spite of the widespread investigation of nitrate intoxication in animals, no study to date has determined the effects of inorganic sulfur supplementation on the thyroid hormones in Angora goats fed with a high-nitrate diet. Therefore, the aim of the present study was to investigate the long-term effects of sulfur supplementation to a high-nitrate diet on thyroid hormones in Angora goats.

MATERIAL and METHODS

Animals

This study was performed on 18 male Angora goats aged 14 months. The study lasted for 180 days. The animals were provided by the Department of Anatolian Agricultural Enterprises Directorate and kept in the farm of the Afyon Kocatepe University Breeding Research Center under the same feeding and maintenance conditions during a 10-day adaptation period. The animals were equally divided into three groups ($n = 6$): control group fed with a basal diet, experimental nitrate group fed with a basal diet supplemented with 1500 ppm nitrate, and nitrate + sulfur group fed with a basal diet supplemented with 1500 ppm nitrate and 1.8% sodium sulfate. The animals were fed twice daily with dry alfalfa at 1% of body weight and 0.57 kg/day concentrate and provided water *ad libitum* through the experimental period. The experimental protocol was approved by the Ethics Committee of the Faculty of Veterinary Medicine (168-AKÜHEK-66-07).

Biochemical Analysis

Blood samples were collected from the jugular vein before the morning feeding on days 45, 90, 135, and 180 of the experiment. Sera were obtained by blood centrifugation (3000 rpm, 10 min, 4°C) and stored at -20°C until analysis. The serum TSH level was estimated using the commercial kits (DiaMetra, Milano, Italy, Ref: DKO013). Serum total triiodothyronine (Diagnostic Systems Laboratories, USA (DSL)-10-3100S), FT₃ (DSL-10-41100), TT₄ (DSL-10-3200), and FT₄ (DSL-10-40100) concentrations were determined by specific enzyme-linked immunosorbent assays.

Statistical Analysis

The statistical differences between the control and experimental groups were evaluated using one-way analysis of variance and Tukey post-hoc tests (SPSS for Windows 11.5.0, SPSS, IL, USA). The data

Table 1. Serum TSH, TT₃, TT₄, FT₃ and FT₄ concentrations during the experimental period in all groups (X ± SEM)

Parameters	Day	Control	Nitrate	Nitrate+Sulfur	P-value
TSH(mlU/L)	45.	2.47 ± 0.95	1.77 ± 0.62	1.81 ± 0.75	0,782
	90.	1.14 ± 0.15 ^b	3.31 ± 0.30 ^a	4.20 ± 0.89 ^a	0,004
	135.	1.33 ± 0.37 ^b	1.69 ± 0.49 ^b	3.98 ± 0.84 ^a	0,014
	180.	1.42 ± 0.44 ^b	1.46 ± 0.46 ^b	3.52 ± 0.61 ^a	0,016
TT ₃ (ng/dL)	45.	103.22 ± 11.49	135.84 ± 19.31	127.49 ± 12.14	0,296
	90.	94.67 ± 8.40 ^b	131.10 ± 14.61 ^{ab}	196.88 ± 29.95 ^a	0,008
	135.	131.75 ± 19.12	115.19 ± 9.61	170.11 ± 16.29	0,065
	180.	112.22 ± 7.62 ^b	107.85 ± 15.94 ^b	193.20 ± 21.87 ^a	0,003
TT ₄ (µg/dL)	45.	25.14 ± 7.40	19.27 ± 3.09	22.51 ± 5.69	0,768
	90.	20.93 ± 4.53	28.75 ± 6.87	14.25 ± 3.79	0,180
	135.	16.00 ± 2.94	19.40 ± 6.74	12.04 ± 2.85	0,533
	180.	27.16 ± 8.16	17.92 ± 3.10	15.34 ± 3.81	0,332
FT ₃ (pg /mL)	45.	2.20 ± 0.12	2.36 ± 0.28	2.26 ± 0.14	0,840
	90.	2.00 ± 0.12	2.36 ± 0.42	3.02 ± 0.43	0,154
	135.	2.13 ± 0.35	2.34 ± 0.12	3.04 ± 0.41	0,138
	180.	2.19 ± 0.10 ^b	2.26 ± 0.20 ^b	3.20 ± 0.42 ^a	0,037
FT ₄ (ng/dL)	45.	0.53 ± 0.07	0.55 ± 0.07	0.70 ± 0.14	0,450
	90.	0.47 ± 0.04 ^b	0.67 ± 0.10 ^{ab}	0.90 ± 0.09 ^a	0,009
	135.	0.50 ± 0.05 ^b	0.71 ± 0.05 ^a	0.84 ± 0.06 ^a	0,002
	180.	0.69 ± 0.10 ^b	0.61 ± 0.08 ^b	1.46 ± 0.13 ^a	0,000

a,b: Differences among groups indicated with different letters in the same row are significant (P < 0.05).

were expressed as mean ± standard deviation. A difference in the mean values with P < 0.05 was considered to be significant.

RESULTS

Data obtained on thyroid hormones during the experimental period are shown in Table 1. On comparing the control group with the nitrate group, serum TSH and FT₄ levels were found to increase on days 90 and 135, respectively. However, no negative effect of nitrate on thyroid hormones was observed in all the groups at the end of the experimental period. On day 180, except for TT₄, the serum TSH and total and free T₃ and free T₄ concentrations were generally higher (P < 0.05) in the nitrate + sulfur group than in the control and nitrate groups.

DISCUSSION

Although the exact mechanism underlying the influence of high nitrate intake on thyroid hormones is unknown, a high nitrate intake by animals may alter the production and secretion of thyroid hormones (Kostogrys et al. 1989). The decreased concentrations of the circulating thyroid hormones may indicate hypothyroidism induced by nitrate toxicity (Jahreis et al. 1986). In the present study, high nitrate intake did not affect the thyroid hormone concentrations in the blood of goats except for samples on day 90 for TSH concentration. The present results indicated that adding 1500 ppm nitrate to diet would not have any negative effects on the thyroid hormones in Angora goats. When the diet contained 1500 ppm nitrate, the nitrate concentration increased in the ruminal fluid, but its concentration remained unchanged in the blood of Angora goats (Ozdemir et al. 2014). This finding suggested that 1500 ppm nitrate could be used as a nitrogen source for protein synthesis by microorganisms in the rumen and had no effect on Na⁺/I⁻ transport protein affecting intrathyroidal iodine concentration (Jahreis et al. 1986, Kostogrys et al. 1989) in Angora goats. In the present study, the blood TT₃ concentration was below the levels (232-252 ng/dL), while the blood TT₄ concentration was above the levels (11.1 and 15.1 µg/dL), reported in Angora goats by previous studies (Keçeci and Keskin 2002, Puchala et al. 2001). The discrepancies in the blood TT₃ and TT₄ concentrations among this and other studies on Angora goats might be due to the differences in the systemic conversion of T₄ into T₃.

The present results showed that sulfur supplementation to a high-nitrate diet increased serum TSH and FT₃ and FT₄ and TT₃ concentrations in Angora goats. Sulfation of thyroglobulin, the thyroid hormone precursor in the thyroid gland, is a major pathway for thyroid hormone synthesis. TSH regulates both thyroglobulin sulfation and thyroid hormone synthesis (Nlend et al. 1999). A previous study (Ozdemir et al. 2014), reported that adding sulfur to a high-nitrate diet decreased the plasma nitrate concentration, but the change was not statistically significant. This suggested that nitrate in the diet with sulfur was increasingly used by microorganisms in the rumen to produce bacterial proteins, and thus sulfur supplementation to a high-nitrate diet decreased the circulating nitrate concentration. However, in the nitrate + sulfur group, an increase in thyroid hormones might be associated with a decrease in plasma nitrate levels due to sulfur. This result indicated that sulfur supplementation to a high-nitrate diet might be an

important factor against the negative effects of nitrate on the production and secretion of thyroid hormones in a dose-dependent manner. No study to date has explored the relationship between sulfur and thyroid hormone metabolism in ruminants. Therefore, changes in circulating TSH, T₄, and T₃ concentrations may not interfere with the ability of sulfur supplementation to stimulate the production and secretion of thyroid hormones in Angora goats.

In conclusion, this study suggested that Angora goats were able to tolerate a feed containing 1500 ppm nitrate with respect to the thyroid hormones and sulfur might serve as a natural source for alleviating the negative effects of the high-nitrate diet on the thyroid gland in a dose-dependent manner. However, more advanced studies are needed to elucidate the relationship between sulfur metabolism and thyroid hormone metabolism.

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