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Effects of supplementation of inorganic sulfur on some biochemical parameters in Angora goat's diet containing high nitrate levels

Mehmet ÖZDEMİR^{1,*}, Abdullah ERYAVUZ², Gülcan AVCI³, Yavuz Osman BİRDANE¹, İsmail KÜÇÜKKURT³ ¹Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey

²Department of Physiology, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey ³Department of Biochemistry, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey

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Abstract: The purpose of this study was to determine the effect of supplementation of sulfur to a diet containing sodium nitrate at a level that would cause chronic nitrate poisoning on some rumen and biochemical parameters in Angora goats. A total of 18 male Angora goats were used. The animals in the control group were fed the basal diet, while the animals were fed experimental concentrate containing 1500 mg/kg sodium nitrate in the nitrate group (N) and experimental concentrate containing 1500 mg/kg sodium nitrate plus 1.8% sodium sulfate in the nitrate-sulfur group (NS) throughout the study, which lasted for 6 months. While there were no differences among groups in live weights, nitrate supplementation to the diet increased the rumen nitrate content and the blood methemoglobin (MetHb) levels and decreased plasma vitamin A and carotene in the N and NS group. The supplementation of sulfur to a diet containing high sodium nitrate alleviated the negative effects of nitrate on the blood MetHb and plasma carotene significantly (P < 0.05). The results of this study suggest that Angora goats can tolerate diets containing the high sodium nitrate levels used in the present study, except for the results in regard to blood MetHb and plasma vitamin A and carotene.

Key words: Angora goat, live weight, methemoglobin, nitrate, sulfur, vitamin A, β-carotene

1. Introduction

Nitrate is not toxic; however, it is converted into nitrite in the body. Therefore, its increased levels in animal feed and water can bring about negative effects on animal health (1). It was reported that nitrogen in nitrate at around 800-2000 ppm in total ingested via feed and water results in subacute and chronic poisoning. The nitrite ion, which is more effective than nitrate, is rapidly ingested throughout the digestive tract, showing its effect by both oxidizing hemoglobin to methemoglobin (MetHb) and relaxing the smooth muscles of the vessels (2). When the density of MetHb, which is normally at the level of 0.5%-3% depending on the animal species, reaches 5%-10%, the initial signs of cyanosis occur (1,3). Additionally, another negative effect of nitrate is that it prevents the conversion of carotenes to vitamin A and reduces its storage in the liver, thereby causing its deficiency in ruminants. The high intake of nitrates and nitrites via diet and water reduce the availability of β -carotene depending on the dose in animals (1,2).

Nitrate poisoning can occur depending on the animal species in different ways (1). In a way that is different

from other animals, ruminant microorganisms reduce the nitrate received via the ration into nitrite. The nitrite is then converted to ammonia and this is used in bacterial protein synthesis. However, rumen bacteria use more ammonia to synthesize the sulfur-containing amino acids in the rumen when rumen sulfur concentration increases. Therefore, the sulfur has an exogenous quality for the sulfurous amino acids to be able to be synthesized by the bacteria in the rumen (4,5). Takahashi et al. (6) reported that the supplementation of sulfur to the diet increases the use of ammonia by the bacteria in the rumen, thereby reducing the accumulation of nitrite. This information indicates that rumen bacteria make a great contribution for preserving the health of the host animal against nitrate poisoning (1). Krasicka et al. (7) showed that the microbial protein synthesis in lambs fed diets containing low cellulose levels increased when the sulfur level was elevated from 0.2% to 0.8% in the dry matter of the ration. Therefore, supplementation of organic or inorganic sulfur compounds into diets containing high levels of nonprotein nitrogen (NPN) is of great importance in terms of ruminant nutrition.

^{*} Correspondence: mozdemir@aku.edu.tr

The objective of this study is to determine the effect of adding sulfur to the diet of Angora goats fed with high sodium nitrate for a long period of time on some rumen and biochemical parameters.

2. Materials and methods

2.1. Animal materials

In this study, 18 male Angora goats aged 14 months with an average weight of approximately 29.5 kg were used. The study animals were bought from the Anatolian Agricultural Enterprises Directorate (Mahmudiye, Eskisehir, Turkey). Throughout the trial period, the animals were accommodated in specially prepared, separate sections at the Directorate for Research Center on Animal Husbandry at Afyon Kocatepe University. The experimental protocol was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Afyon Kocatepe University. Before the trial commenced, random sampling was performed at the end of a 10-day long adaptation period and the animals were divided into 3 groups with 6 experimental animals per group. The first experimental day the animals were weighed to check their live weights.

The animals were divided into 3 groups as follows: the control (C) group was fed with a basal diet; the nitrate (N) group of animals was fed with concentrate containing 1500 ppm sodium nitrate, reported to cause chronic poisoning (2); and the nitrate-sulfur (NS) group of animals was fed with 1500 ppm sodium nitrate as well as sodium sulfate added at the rate of 1.8% of concentrate (4).

Throughout the study, the animals were given dry alfalfa at a rate of 1% of their body weight and 0.57 kg/

day of concentrated feed (8). The animals were fed with the composed feed for the control and study animals as shown in Table 1 throughout the study. The dry alfalfa and concentrate feed were given in the morning and evening in 2 equal meals; the dry alfalfa was given first and it was followed by concentrate feed in order. Throughout the trial, clean drinking water was continuously provided for the animals.

2.2. Sampling and biochemical analysis

The live weights of animals were measured at the onset and at monthly intervals. Throughout the 6 months of study in total, blood and ruminal fluid samples were collected on days 45, 90, 135, and 180. The pH, nitrate, nitrite, and ammonia levels in the rumen fluid samples and the plasma urea, total protein, MetHb, vitamin A, β -carotene, nitrate, and nitrite levels in the blood samples were measured.

The samples from the rumen fluid were collected on every sampling day, 4 h after the morning feed and following massage on the rumen sites of animals. They were obtained from the ventral rumen sac by means of a probe with an inner diameter of 5–6 mm and a plastic injector of 50 mL. As for the blood samples, they were collected in sufficient amounts from the jugular vein at the same periods. Following the collection of blood samples, the serums and plasmas were immediately separated; they were protected against daylight and stored in deep freeze at –20 °C until they were analyzed.

The nitrate and nitrite levels in the rumen fluid were measured using the method reported by Starh (9) and the plasma nitrate and nitrite levels were measured using a spectrophotometric method reported by Schneider

Ingredient composition (%)	C group	N group	NS group
Corn	30.76	30.76	30.76
Barley	15.00	15.00	15.00
Sunflower seed meal	18.89	18.74	16.94
Wheat - middling	25.00	25.00	25.00
Molasses	7.18	7.18	7.18
Limestone	2.23	2.23	2.23
Salt	0.84	0.84	0.84
Vitamin - mineral premix [#]	0.1	0.1	0.1
Sodium nitrate	-	0.15	0.15
Sodium sulfate	-	-	1.8

Table 1. Composition of the concentrates fed to animals in control and experimental groups.

[#]Vitamin mineral premix [Rovimix 302 (Roche)] provided per kilogram of diet: vitamin A, 15,000,000 IU; vitamin D_3 , 3,000,000 IU; vitamin E, 30,000 mg; Mn, 50,000 mg; Fe, 50,000 mg; Zn, 50,000 mg; Cu, 10,000 mg; iodine, 800 mg; Co, 150 mg; Se, 150 mg.

and Yeary (10). The levels of ammonium nitrogen in the rumen fluid (BioSystems, Spain, Cat. No: 11536), nitrogen in the plasma urea (BioSystems, Cat. No: 11536), and total protein (Chema Diagnostica, Italy, Cat No: TP500CH) were determined spectrophotometrically using commercially available test kits. The pH values of rumen fluid samples were promptly measured using a digital pH meter (WTW 330). The serum vitamin A and β -carotene levels were determined spectrophotometrically using the method reported by Suzuki and Katoh (11). The blood MetHb levels were identified spectrophotometrically according to the method reported by Fairbanks and Klee (12).

2.3. Statistical analysis

The derived data were assessed using SPSS 11.5.0. The data are reported as mean \pm standard deviation values. Normality tests were conducted on the data obtained; the ANOVA test was used to identify the statistical differences among groups and the Tukey test was conducted as a posttest.

3. Results

The average live weights of experimental animals are specified in Table 2. The mean values and standard deviations pertaining to the pH, nitrate, nitrite, and ammonium nitrogen levels of the rumen fluid are stated in Table 3. The mean values and standard deviations pertaining to the plasma urea N, total protein, MetHb, vitamin A, β-carotene, nitrate, and nitrite levels identified in the blood samples are shown in Table 4. While there were no differences among groups in live weights, sodium nitrate supplementation to concentrate increased the rumen nitrate content and the blood MetHb levels and decreased plasma vitamin A and β -carotene in the N and NS groups. The supplementation of sulfur to a diet containing high sodium nitrate alleviated the negative effects of nitrate on the blood MetHb and plasma carotene significantly.

4. Discussion

The sulfur requirements of ruminants increase when their diets contain NPN (5). The rumen microorganisms can obtain sulfur in 2 ways: breakdown of ration proteins or addition of inorganic sulfur to diet. It was reported that rumen bacteria have more sulfur-containing amino acids than the other microorganisms in the rumen (4-6), that the nitrogen/sulfur ratio of bacteria in the rumen is 16/1 (13), and that in vitro addition of sulfur increases the cysteine level of bacteria (14). The US National Research Council (NRC) (15) reported the required nitrogen/sulfur ratio in the ration of ruminants to be 10/1 for goats. Erdinc (4) showed in a study conducted on sheep fed with ration containing 3% urea into which sodium sulfate was added at various levels that the highest live weight increase was obtained in the group that had the 1.8% sodium sulfate added to their ration. Departing from this, in the present study, the nitrogen/sulfur ratio of the control ration was kept at the level reported by the NRC (15) while the nitrate-containing ration was enhanced with 1.8% sodium sulfate as a source of sulfur in order to assure that the rumen microorganisms could benefit further from the nitrogenous matters in the ration.

There were no differences in the live weights among groups recorded on the days of weighing. In groups C, N, and NS, the mean live weights obtained at the end of the 180-day-long research period were 43.57, 45.38, and 44.08 kg, respectively (Table 2). This result is compatible with reports that there were no changes in live weight among cattle fed with rations containing high levels of nitrate (16). The result obtained in the NS group is also consistent with reports that the addition of sulfur to the ration did not affect the live weights of Angora goats (13).

The ruminal pH levels were increased by supplementation of sodium nitrate to concentrate in this study. This result, which is observed to be compliant with the reports of an increase in the rumen pH throughout a

Table 2. Mean	live weights of	f animals in c	ontrol and e	experimental	groups.

		C group	N group	NS group	P-value
	Initial	28.50 ± 1.74	30.27 ± 2.79	29.97 ± 1.74	0.826
	1st month	31.32 ± 1.83	32.83 ± 3.06	32.55 ± 2.35	0.899
	2nd month	32.02 ± 1.90	33.40 ± 3.24	33.38 ± 2.78	0.917
Live weight (kg)	3rd month	35.55 ± 2.59	36.88 ± 3.46	36.15 ± 2.97	0.953
	4th month	41.12 ± 3.21	42.50 ± 4.02	41.65 ± 3.49	0.963
	5th month	42.13 ± 3.17	43.52 ± 3.64	42.72 ± 3.60	0.961
	6th month	43.57 ± 3.40	45.38 ± 3.47	44.08 ± 3.67	0.932

Parameter	Measurement time	C group	N group	NS group	P-value
	1	$7.08\pm0.05^{\rm ab}$	$6.97\pm0.12^{\rm b}$	$7.29\pm0.03^{\text{a}}$	0.027
	2	$6.95\pm0.08^{\rm b}$	$7.06\pm0.08^{\rm b}$	$7.45\pm0.04^{\text{a}}$	0.006
pН	3	$6.97\pm0.12^{\rm b}$	$7.54\pm0.08^{\rm a}$	$7.31\pm0.05^{\text{a}}$	0.002
	4	7.10 ± 0.08	7.24 ± 0.07	7.41 ± 0.10	0.056
	1	$0.238\pm0.05^{\mathrm{b}}$	$0.850\pm0.09^{\rm a}$	$0.703\pm0.08^{\rm a}$	0.000
	2	$0.235\pm0.03^{\mathrm{b}}$	$0.665\pm0.05^{\rm a}$	$0.567\pm0.03^{\text{a}}$	0.000
Nitrate (ppm)	3	$0.308\pm0.04^{\rm b}$	$0.624\pm0.03^{\rm a}$	0.563 ± 0.01^{a}	0.000
	4	$0.366\pm0.03^{\rm b}$	$0.769\pm0.04^{\rm a}$	0.661 ± 0.05^{a}	0.000
	1	0.137 ± 0.02	0.148 ± 0.02	0.129 ± 0.03	0.738
	2	0.111 ± 0.01	0.169 ± 0.02	0.144 ± 0.01	0.079
Nitrite (ppm)	3	0.133 ± 0.02	0.331 ± 0.08	0.285 ± 0.06	0.070
	4	0.218 ± 0.02	0.320 ± 0.04	0.273 ± 0.04	0.132
	1	57.69 ± 4.97^{a}	$43.18 \pm 1.31^{\rm b}$	$38.54 \pm 1.33^{\mathrm{b}}$	0.001
NH ₃ -N (mg/dL)	2	30.53 ± 4.55	39.38 ± 3.50	29.84 ± 3.92	0.207
	3	37.23 ± 6.21	37.79 ± 3.41	35.29 ± 2.21	0.911
	4	39.30 ± 0.10	38.07 ± 0.62	38.57 ± 0.35	0.138

Table 3. Mean values of some ruminal fluid parameters in experiment.

a, b: Differences among groups indicated with different letters in the same row are significant.

Measurement times: 1 - day 45, 2 - day 90, 3 - day 135, 4 - day 180.

24-h observation following the administration of nitrate, can be attributed to the increased amount of ammonia as a result of the reduction of nitrate and the decreased cellulose digestion in the rumen due to nitrate (17). In fact, Spears et al. (18) found that nitrate decreased cellulose digestion and that the addition of sulfur to a nitrated environment increased cellulose digestion. However, this increase was lower as compared to the addition of sulfur into an environment without nitrate. This result in the present study suggests that nitrate may also reduce the cellulose digestion, thereby contributing to the maintenance of a high pH level in the rumen. The fact that the addition of sulfur to a ration with high nitrate content raised the pH level significantly as compared to the control group seems compatible with the report that the addition of sulfur to the ration of Angora goats increased the value in the mentioned parameter (13).

There are no differences in nitrate levels of the rumen fluid among groups N and NS in the present study, whereas the values in both groups were higher than in group C at all sampling times. This result is in conformity with the report that increased nitrate level in the ration also increases the nitrate level in the rumen (19). It is not consistent with a report that the nitrate level in the rumen returned to the level it was at before administration and 7 h following its administration (6). There are different reports from studies conducted on the nitrate level in the rumen. These differences might be attributed to the experimental animals used in the studies, ration compositions, amounts of nitrate and their modes of administration to animals, or adaptation of animals to nitrate-containing rations, as well as differences in sample collection times (1). The fact that there were no differences between groups N and NS with regards to the mentioned parameter is similar to the finding that the addition of L-cysteine to a ration containing a subclinical level of nitrate did not affect the nitrate level in the rumen (6).

Nitrate is more rapidly reduced to toxic nitrite in the rumen by bacteria; the nitrite is absorbed more rapidly from the rumen and transmitted to the blood (2). There were no statistical differences among groups with respect to nitrite levels (Table 3). Burrows et al. (17) reported that the nitrite levels in the rumen reach a peak at 6 to 8 h after nitrate is administered to the rumen and that they return

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Parameter	Measurement time	C group	N group	NS group	P-value
	1	$48.78\pm2.18^{\rm b}$	$51.40 \pm 2.00^{\mathrm{ab}}$	58.64 ± 2.71^{a}	0.024
	2	56.30 ± 3.44	58.87 ± 3.76	50.42 ± 1.38	0.167
Urea-N (mg/dL)	3	50.68 ± 4.28	48.54 ± 1.29	48.64 ± 2.64	0.851
	4	42.42 ± 1.97	44.83 ± 1.78	44.00 ± 1.76	0.648
	1	7.12 ± 0.19	7.08 ± 0.12	6.41 ± 0.19	0.165
	2	6.79 ± 0.15	6.71 ± 0.18	6.37 ± 0.12	0.147
Total protein (g/dL)	3	7.00 ± 0.15	6.98 ± 0.23	6.81 ± 0.12	0.713
	4	7.12 ± 0.10	7.33 ± 0.23	7.31 ± 0.21	0.695
	1	$2.54\pm0.07^{\rm b}$	$3.60\pm0.26^{\rm a}$	$3.14\pm0.16^{\text{ab}}$	0.003
	2	$2.74\pm0.10^{\rm b}$	$3.59\pm0.24^{\rm a}$	$3.19\pm0.06^{\text{ab}}$	0.006
Methemoglobin (%)	3	$2.75\pm0.11^{\mathrm{b}}$	3.68 ± 0.12^{a}	$3.13\pm0.07^{\rm b}$	0.001
	4	$2.76\pm0.09^{\rm b}$	$3.60\pm0.13^{\text{a}}$	$3.08\pm0.07^{\rm b}$	0.000
	1	46.01 ± 5.22^{a}	23.64 ± 2.21^{b}	$15.95 \pm 1.02^{\text{b}}$	0.000
57° · A (/ IT)	2	$33.40\pm2.57^{\rm a}$	26.10 ± 1.43^{b}	$21.04\pm0.54^{\rm b}$	0.001
Vitamin A (µg/dL)	3	$48.85\pm8.64^{\rm a}$	17.28 ± 1.53^{b}	19.56 ± 1.19^{b}	0.001
	4	$23.31\pm0.64^{\rm a}$	$16.02 \pm 0.62^{\circ}$	$21.08\pm0.50^{\rm b}$	0.000
	1	$2.52\pm0.12^{\rm a}$	$2.51\pm0.10^{\rm a}$	1.61 ± 0.19^{b}	0.000
	2	2.60 ± 0.12^{a}	$1.59\pm0.12^{\rm b}$	$1.91\pm0.18^{\rm b}$	0.001
3 carotene (μg/dL)	3	$5.49 \pm 1.09^{\text{a}}$	$1.29\pm0.13^{\rm b}$	$3.10\pm0.69^{\text{ab}}$	0.004
	4	$4.27 \pm 1.00^{\text{a}}$	$1.10\pm0.15^{\rm b}$	$2.26\pm0.60^{\rm ab}$	0.016
	1	$0.441\pm0.04^{\rm b}$	0.802 ± 0.11^{a}	$0.752\pm0.09^{\rm ab}$	0.025
	2	$0.478\pm0.05^{\rm b}$	$0.680\pm0.06^{\mathrm{a}}$	0.523 ± 0.02^{ab}	0.021
Nitrate (ppm)	3	0.370 ± 0.03	0.454 ± 0.04	0.424 ± 0.03	0.256
	4	0.255 ± 0.02	0.334 ± 0.04	0.301 ± 0.04	0.327
Nitrite (ppm)	1	0.150 ± 0.03	0.235 ± 0.09	0.183 ± 0.09	0.254
	2	0.315 ± 0.06	0.458 ± 0.07	0.396 ± 0.03	0.227
	3	0.276 ± 0.08	0.418 ± 0.10	0.377 ± 0.08	0.523
	4	0.223 ± 0.04	0.283 ± 0.04	0.267 ± 0.04	0.596

Table 4. Mean values of some blood parameters related to nitrogen and sulfur feeding.

a, b: Differences among groups indicated with different letters in the same row are significant. Measurement times: 1 - day 45, 2 - day 90, 3 - day 135, 4 - day 180.

to baseline levels 12 h later. The fact that there were no statistical differences among groups with respect to nitrate levels even though the rumen nitrate level in this study was high in groups N and NS at the same measurement time can be attributed to the fact that the nitrite level was measured in samples taken shortly after the feeding. On the other hand, the rumen nitrite levels obtained for all groups are close to the values reported for cattle by Oruç and Ceylan (20) (0.31 ppm) and consistent with the values reported in sheep by Eryavuz et al. (5) (0.06–0.31 ppm).

While the rumen fluid ammonia nitrogen levels identified throughout the study were significantly different between group C and the other groups (P < 0.001), the differences among groups were insignificant in the consequent periods. The value obtained in group NS was consistent with the report that the addition of sulfur to the ration did not affect the ammonium level of the rumen (21). It was reported that the extent to which the bacteria reduce nitrite to ammonia may be reduced if the level of ammonia in the rumen is high (22). Taking this situation into account, the fact that the groups do not have any differences with respect to ammonium nitrogen suggests that the level at which nitrite is reduced to ammonium is low since the bacteria meet their required level of ammonium nitrogen.

There were no differences in plasma urea levels among groups in the present study. Plasma urea nitrogen is formed either as a result of the conversion into urea of ammonia, which occurs in the liver as a result of the breakdown of nitrogenous substances or body proteins for generating energy, or as a result of the absorption and conversion into urea of the ammonium, which occurs after the breakdown of nitrogenous substances in the rumen and is excessive for use by bacteria (5). The mentioned values identified in all groups in the study are higher than the values of 12-18 mg/dL reported by Harris (23) for dairy cattle. It was reported that a plasma urea concentration below 12 mg/dL is due to the low level of crude protein in the ration or low amount of digestible protein in the rumen, and levels above 18 mg/dL are due to excessive amounts of crude protein or digestible protein in the rumen or low amounts of energy fermentable in the rumen, or an imbalance between those (23). On the other hand, it was reported that goats have higher plasma urea nitrogen than cattle and sheep (24); however, the results in this study are higher than the values reported for Angora goats (25). The reason for the discrepancies between these reports could be the differences among sources of nitrogen and energy constituting the ration composition used in the studies (23).

There were no differences in plasma total protein among groups in the present study and the values were in the normal ranges (5.60–7.50 g/dL) reported for ruminants (26).

The supplementation of sodium nitrate to concentrate increased the MetHb levels in the goats. This result was found to be consistent with several reports indicating that the MetHb levels were increased in ruminants fed with ration and water containing high amounts of nitrate (2,6). On the other hand, the fact that the values obtained in group NS were found to be statistically insignificantly higher than those obtained in group C yet significantly lower than those for group N in the last 2 sampling periods was consistent with the report that the addition of a source of sulfur to the ration reduced the MetHb level (6). The mentioned values obtained for group C in the study are compatible with the values that are reported to be generally 1%–3%, although the normal blood MetHb level varies (2). The values obtained for groups N and NS were slightly above the mentioned levels; however, the clinical signs were quite below the levels reported (30%-40%) (1).

Compatible with the reports that vitamin A deficiency was observed in ruminants consuming ration or water with high nitrate content, it was observed that the values obtained for groups N and NS were lower than those obtained for group C at a statistically significant level (P < 0.001) in all sampling periods. This study indicates that the addition of sulfur to concentrate with high sodium nitrate content does not have an effect of correcting the negative effect of nitrate on vitamin A. The values obtained for the control group were found to be within the normal ranges reported by Altıntaş and Fidancı (26) for sheep (20–45 µg/dL) and close to the values (41 µg/dL) they reported for goats, while it was observed that the values obtained for groups N and NS were close to or below the lower threshold of normal values reported for sheep.

It was found in this study that the values obtained for β -carotene in groups N and NS were statistically significantly lower than those obtained in group C at the first sampling time (P < 0.001), and that the values for group N were significantly lower than those for group C and the values for group NS were significantly lower than those for group C at the last 2 sampling times (Table 4). This result was consistent with a report that nitrate limited the conversion of carotenes to vitamin A in the liver, which in turn reduced the storage of this vitamin in the liver (2). On the other hand, the fact that the plasma β -carotene and vitamin A levels obtained in groups N and NS were within the ranges reported for Angora goats (1.17-6.63 and 13.44-27.05 µg/dL, respectively) (27) indicates that a ration containing nitrate at the level used in this study would not cause any significantly negative effects in the metabolism of vitamin A in Angora goats.

The plasma nitrate levels in groups C, N, and NS throughout the study were found to be in the ranges of 0.255–0.478, 0.334–0.802, and 0.301–0.752 ppm, respectively. These values that were obtained are above the value reported by

Oruc and Cevlan (20) for cattle they fed with normal rations (0.13 ppm) and guite below the levels reported by Boermans (28) for cattle showing signs of acute nitrate poisoning (97.4-184.3 ppm). The plasma nitrite level reflects the level occurring as a result of both the oxidation of nitrite absorbed from the rumen into nitrate (29) and the oxidation of NO generated from L-arginine both endogenously and via oxide synthesize enzyme first into nitrite than into nitrate (30). The plasma nitrate level obtained in group N in this study is generally higher than the ones obtained for group C, with the value at the first sampling time being significantly higher (Table 4). Although the addition of sulfur into a concentrate containing sodium nitrate decreased the mentioned value. it was found to be higher than that of group C even though it was not statistically significant (P > 0.05). This is an indication that a ration containing the level of nitrate used in this study would increase the plasma nitrate level and that the sulfur might still remain high even if it reduces the plasma nitrate level.

It was found in this study that were no statistically significant differences among groups with respect to the plasma nitrite level obtained at all sampling times; however, the values obtained for groups N and NS were generally higher than those obtained for group C (Table

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4). This result can be explained by a report that the nitrite transmitted into the blood stream is again oxidized to nitrate while the nitrate that has emerged cannot again be reduced to nitrite and eliminated in urine (29). It was stated that the half-life of NO endogenously synthesized in some tissues is very low and that it is oxygenated initially to nitrite, then to nitrate, and eliminated from the body in urine (30).

In conclusion, the increased plasma MetHb and rumen nitrate levels and the decreased vitamin A and β -carotene levels indicate the negative effects of nitrate in the concentrate; however, no changes were seen in the other parameters that were investigated, which shows that Angora goats are able to tolerate feed containing nitrate at this level. On the other hand, sodium sulfate added to the concentrate especially reduces MetHb and increases vitamin A, which is an indication that sulfur in the concentrate has beneficial effects against the negative effects of nitrate.

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